RESEARCH REPORT

1.Introduction.

I am still focussing on the development of rapid techniques to detect point mutations in mammalian cells. There are at least two categories of point mutations; (1) those for which you already know the DNA sequence change, and (2) those that might be somewhere in the gene that you are interested in, but you are not sure where. Point mutations that are in the first category include the human hemoglobinopathies (sickle cell anemia, etc), alpha-1-antitrypsin deficiency and HLA alleles. Point mutations in the second class include the newly arising point mutations responsible for some of the Lesch-Nyhan cases in our collection and (probably) some of the mutations leading to DMD.

Point mutation detection techniques can be divided into a similar two categories; (1) techniques that are only useful if you already know what the mutation is, or (2) Procedures that can 'scan' a large length of DNA or RNA for a single base change. Allele Specific Oligonucleotide (ASO) probing is a procedure for identifying well characterised point mutations. RNase A cleavage, denaturing gradient gel electrophoresis and the strand-displacement assay (Figure One) are all procedures for 'scanning' for new mutations.

Each of these procedures can be used in conjunction with the polymerase chain reaction (PCR) procedure, which can amplify target sequences and simplify the subsequent analyses. PCR has also opened up the possibility of direct DNA sequencing, either to detect a previously known mutation or to look for new ones. In this report I will describe a number of different experimental -point mutation detection systems that I have been exploring. Almost all of these are PCR based. They include denaturing gradient gel electrophoresis and direct sequencing strategies (with Richard Blaszak), and point mutation detection by competitive oligonucleotide priming (with Nikki Nguyen).

2. Optimising PCR Reaction Conditions.

We now have quite a bit of experience with the 'basic' PCR reaction. At first the reaction did not work at all, but fiddling with several variables has identified conditions that give consistently good amplification. Because there are a large number of variables, most results are 'anecdotal'. Below are the final conditions that we arrived at for amplifying fragments of 30

the internal probes. Good signals were not obtained. Sometime was spent determining good conditions for blotting small fragments (Figure Five) and we found that a 1.5% agarose gel, transfered to to 'Zeta-Probe' in o.4N alkali gave a substantially better result than NuSieve and/or genesreen.

Meantime, we also found that primer extension provided an easier and more sensitive assay for the PCR products (Figure Six). We have since avoided the filter hybridisations wherever possible. The identification of DMD deletions by this method is shown in Figure Seven.

4. PCR and Direct DNA Sequencing.

If you can identify a PCR internal primer-extension product on a gel, then you are close to a DNA sequencing strategy. We have tried several times to break down a primer extension product with a dideoxy chain termination ladder, but have not yet done so. An example is shown in Figure eight. There is now published one example of direct DNA sequencing of PCR products from mammalian cells, and we note that they use 36 rounds of PCR followed by a gel purification step. These experiments are ongoing. We emphasise that although we feel that the direct sequencing will be a powerful tool, it will be no means preclude the use of other mutation detection techniques.

5. PCR and Denaturing Gradient Gel Electrophoresis.

The primer extension products mentioned above are suitable for analysis on denaturing gradient gels. We have the gel system running, as shown in Figure 9. So far primer extension products have not yielded products with good melt-transitions (see overhead). We believe this to be due to the short length of the duplexes that we have analysed. We intend to test out longer duplexes.

6. Competetive Oligonucleotide Priming

It is well established that oligonucleotides will bind to complementary DNA even when the homology is not complete. This is the basis of the ASO detection system, and is frequently used in PCR reactions and in site directed mutagenesis strategies. We have found, quite surprisingly, that when two oligonucleotides are supplied to a hybridisation reaction, at low stringency, then a 100% match is strongly favoured over a single base mismatch. We have demonstrated this with several different primers and

C. Flourescent Probes

We are collaborating with Ken Beatty to use the competeive oligo priming system with flourescently labelled oligo's to do simple detection of human disease mutations. Towards this end, I have synthesised oligos with a 5' aliphatic amino group, using commercially available witchcraft, and Ken Beatty has conjugated these to flourescent dyes. The relevant structures are in Figure Twelve. This strategy is being piloted on the *spf* cloned cDNA with flousescein and Texas Red tagged primers.

D. Next

The competetive priming system is ready to try on genomic DNA with flourescent primers. As an added refinement we are trying to construct a solid support for the 'universal' primer, to allow the thing to be automated.

6 Scheme To Revolutionise Mutagen Screening

Finally, I would like to discuss an idea that Grant MacGregor and I have conceived for mutagen screening in mammalian cells. The strategy is shown in Figure Thirteen. The advantages of this scheme are that no selection need be imposed on the mammalian cells and that the precise DNA sequence changes that are induced can be readily obtained. The scheme will take advantage of an on-line ^{32}P detector that can do automated DNA sequencing. This machine is made by a company called EG and G, based in Boston. I have initiated a agreement for them to lend us a machine for two months to try some automated mutation detection strategies. One glitch in the scheme is that the background in the plasmid ligations may be high, but we are optimistic.

7. Summary

We are continuing to develop strategies for point mutation detection. Most are PCR based. PCR plus DNA sequencing and PCR plus denaturing gradient gel electrophoresis are almost working. A new point mutation detection strategy has been developed, based on competition between priming oligonucleotides. Competetive oligonucleotide priming has the potential to replace ASO probing for the detection of previously characterised point mutations.

0349R

RESEARCH REPORT

Introduction

My main aim is to develop improved methods for detection of point mutations in mammalian cells. The techniques being used are ribonuclease A (RNase A) cleavage, denaturing gradient gel electrophoresis, and the polymerase chain reaction (PCR) procedure for the amplification of specific nucleotide sequences. HPRT is serving as a 'model locus' for development of the procedures.

Ultimately we want rapid procedures with the power for the detection of all possible point mutations. Neither RNase A cleavage nor denaturing gradient gel electrophoresis are capable of this in their current form. These procedures also share a problem with Southern and Northern blotting- unique mammalian DNA sequences are rare and difficult to detect. PCR offers an approach to alleviate this limitation.

In this report I will describe a procedure for the detection of point mutations using denaturing gradient gel electrophoresis and PCR, that is under development. I am focusing on this combination of procedures because they offer, for the first time, the potential to detect all point mutations in a simple, rapid, easy manner. Several other strategies for 'molecular diagnostics' using denaturing gradient gels, RNase A cleavage and PCR have been conceived. Some have been explored and will be discussed. The end of the spf mouse study will also be described. Finally, much of this work is dependent on the availability of synthetic oligonucleotides. Our new synthesiser will be up on the 26th of May, and interested people can start placing orders.

Denaturing Gradient Gel Electrophoresis and PCR with polyC Tails.

Denaturing gradient gel electrophoresis can resolve point mutations in double stranded DNA due to the resultant differences in thermal melting temperatures (Tm's) of the duplexes. Because it is the formation of partially denatured molecules which results in changes in electrophoretic mobility, only single DNA base changes which are within the 'melting domains' with the lowest Tm's in DNA duplexes will be detected. Myers et al. have demonstrated that when a duplex is covalently linked to a highly GC rich DNA sequence then the probability of detecting point mutations is increased. This is because the GC 'clamps' have a relatively high Tm, and so all the rest of the duplex must fall into a relatively low Tm region. Myers et al. used the approach of cloning the sequence to be analysed into a vector containing the beta-globin promoter, in order to introduce the GC clamps. This is too cumbersome for routine analysis of mammalian genes. Therefore an alternative approach to introduce the GC clamps will be employed. PolyC tails which are present on the 5' end of oligonucleotides will be introduced into PCR amplified HPRT sequences. The opposing primer for the reaction will be have a ^{32}P end label. After PCR, the sequences of interest will be amplified, labelled and attatched to the GC clamp. These can be loaded directly onto a denaturing gel or alternatively, cut out of a NuSieve agarose gel and then run on the denaturing gel.

Progress towards having this procedure up and running has been directed towards getting the PCR going and trying out the denaturing gradient gels. The PCR works fine on cloned DNA (second figure), using HPRT cDNA primers. PCR of exon 9 HPRT sequences and beta globin DNA sequences have both shown a band visible by ethidium bromide staining after twenty reaction rounds

(third figure). The denaturing gradient gels have been run with 'perpendicular' gradients, and two RNA:RNA hybrids generated from HPRT cDNA cloned into pTZ. Interesting patterns have emerged. The denaturing gradient gels were quite simple to form and run. Currently the limiting factor is oligonucleotides, which have been on order for some time. It is possible that the polyC tailed oligos will pose problems with the synthesis. Once our machine is running I will synthesise a stock of $(C)_n$, and then attempt to ligate this to other oligos using a GGGGNNNN 'splint'.

PCR Diagnosis of DMD

Now that DMD gene sequences are available it is possible to try using some new 'tricks' to speed up DMD diagnosis. Oligos spanning short regions of DMD exons (from within pert 87.4 and 87.25) have been obtained. A high proportion of DMD cases are deleted for these sequences (5 - 20%). In these cases there will be no DNA sequences to prime, therefore no PCR reaction product. HPRT/beta globin/alpha 1 antitrypsin primers can serve as internal controls and amplified products can be identified on agarose gels, or alternatively in a Southern blot or in a series of dot blots with oligos which are internal to the PCR primers.

Sparse Fur OTC

Since Steves last talk, we have conducted a functional analysis of the mutation that we have cloned from the spf mouse. We find that the cloned sequence mimics the previously reported pH dependent change in activity optima that was reported in the original spf mouse. Therefore, we believe that we cloned out the real thing and not some terrible artifact.

Conclusion

RNase A cleavage is no longer the primary focus for mutational analysis. PCR and denaturing gradient gel electrophoresis are currently favored as the best combination of techniques for detecting most point mutations. PolyC tailed oligonucleotide primers are about to be used to directly introduce GC clamps into PCR amplified DNA sequences. The spf mutation seems to have been identified correctly.

1. It is well documented in several families that a new mutation deletion in the DMD locus was recurrent from a mother who had no somatic tissue evidence of the deletion. Presumably these mothers are gametic mosaics for the deletion. The frequency is unknown. I feel all our deletion cases should be reviewed (J.W.) for this setting and revised counseling (P.W.) provided. PND appears indicated until the risk is better documented. Conservative view.

2. The time is ripe to initiate a paper on our deletion cases (J.W.). This data is being put together by A. Roses and I'm sure others. I can help in the case identification 1-200. Beyond this point a review of each report is indicated.

4. I am pleased we can proceed with DMD mouse paper. I suggest a table of RFLPs be added. If we have used Hind III, the gene position of the RFLP can be determined and stated. "Science" is the Target (JC) and submission should follow the L. Kunkel submission to "Cell" - soon. I now think mdx may be Beckers and urge that we examine all independent mdx for exon deletions with our mouse cDNA and consider RNAse A after Northers have been run. Jeff, I think you are logical for this but let's discuss.. To further refine the map position - I will write S. Orkin for CGA probe. We should keep in touch with Ed McCabe on Gkinase. I'd like to use H. Moser's AdLDys probe (T.W.). This may make the present animals more informative. For future animals I feel survey of 100-200 males from MDXC57B/sp with G6PDH/CGP/DMD/G/K/α Galactosidase/ADLD/FACTOR8 is indicated. MDA will accept a grant for this study of MDX/DMD. I need to discuss with Jeff and Tom. Verne Chapman may be sensitive to our doing these studies. We will need to discuss with him.

7. I doubt that L.Kunkel will provide us with materials for simplifying diagnostics. He may try to do it himself. I feel an effort at PCR for the exon used by Jeff to identify our clonses is indicated. The region is small. The flanking sequence is published. We have mutants to test the idea. I'd like to discuss this with Jan and Richard. The long range goal would be a dot/blot for deletions which could span the gene but not examine all exons. The first to establish the principle will lead. MDA will accept a Task Force application on this approach.

C. Thomas Caskey, M.D.

CTC:htj

FIGURE SIX

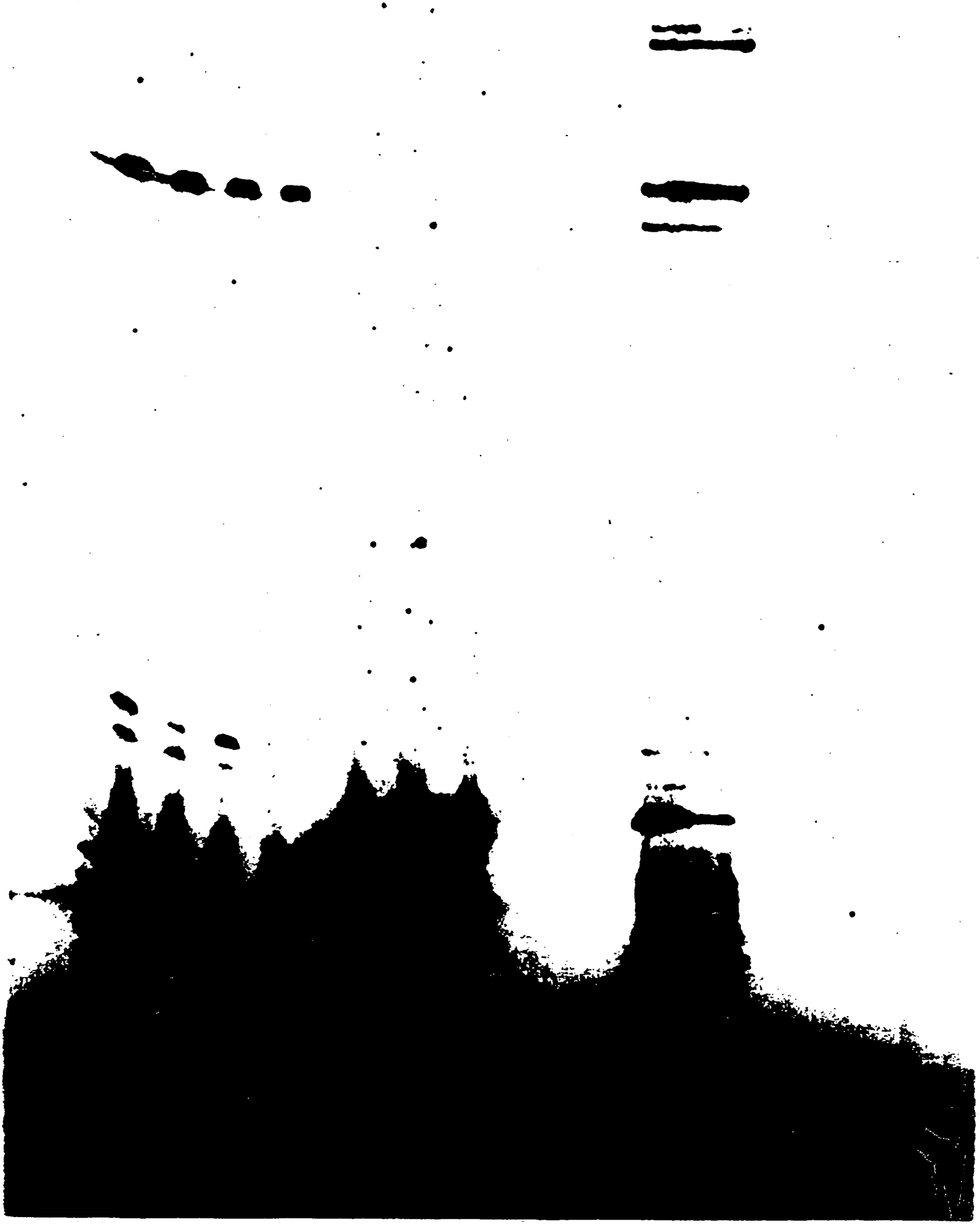


FIGURE EIGHT

mp18 Current length: 130 ENTRY: Displa

```
                                                    SacI  RsaI  MspI
        10         20         30         40         50         60
CACACAGGAA ACAGCTATGA CCATGATTAC GAATTCGAGC TCGGTACCCG GGGATCCTCT

        70         80         90        100        110        120
AGAGTCGACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG

       130        140        150        160        170        180
GGAAAACCCT
```

primer families.

*universal primer.

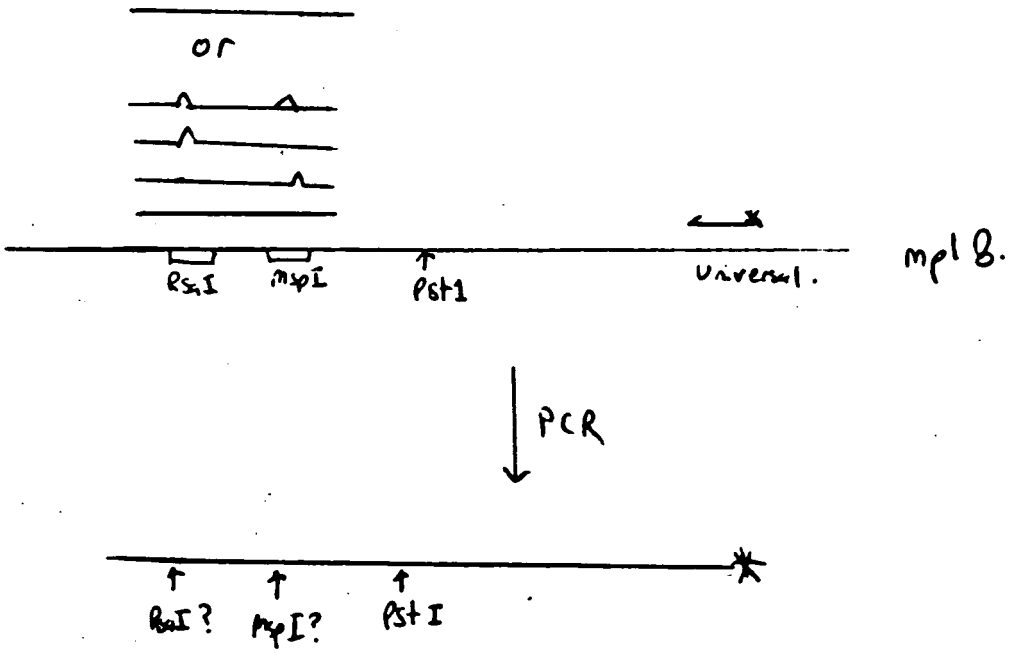
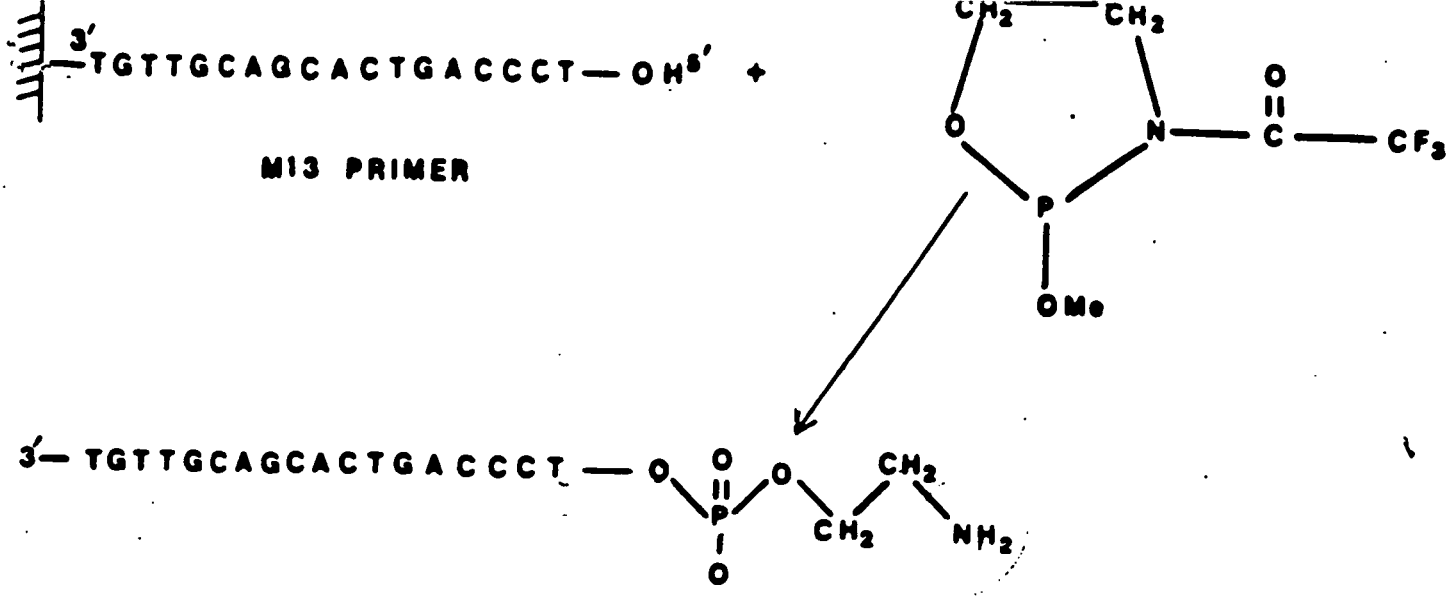


Figure 10.



AMINOLINK—OLIGONUCLEOTIDE PRIMER

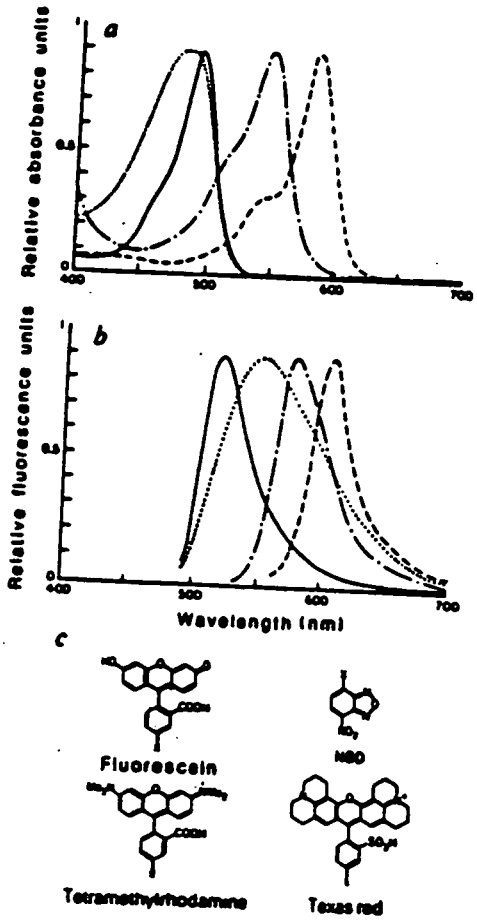
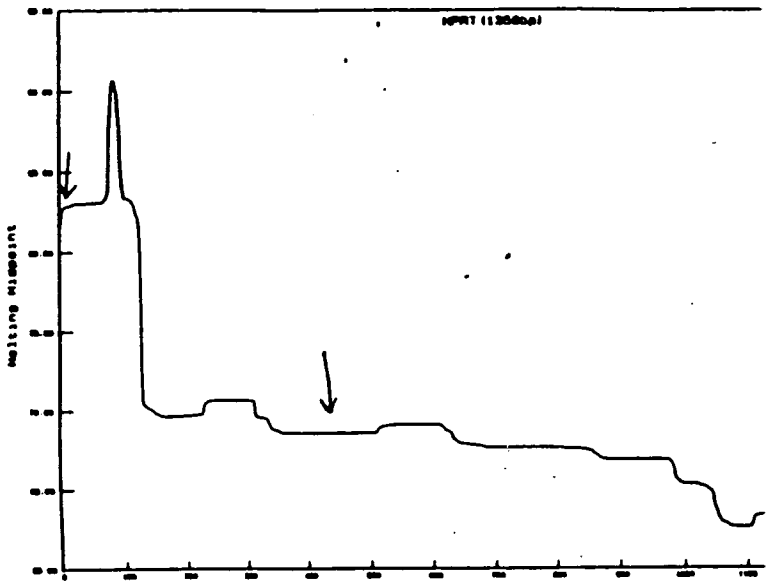
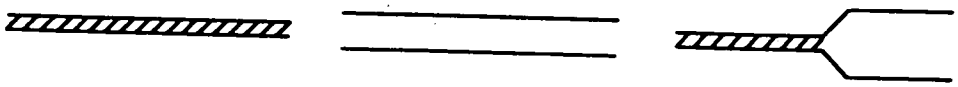
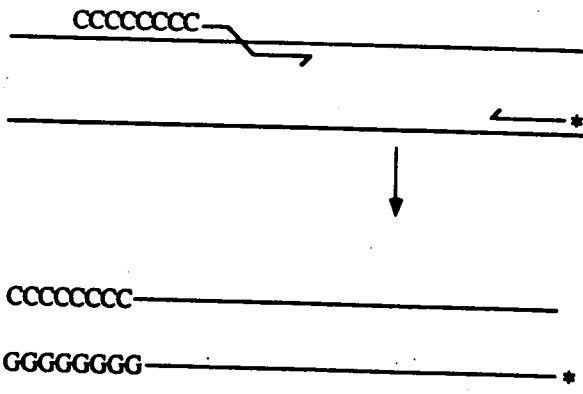


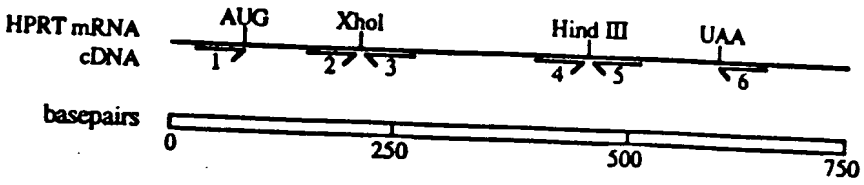
Fig. 2 *a*, Absorption spectra of the four dyes used in the DNA sequenator: ——, fluorescein; ·····, NBD; -·-·-, tetramethylrhodamine; - - - -, Texas Red. *b*, Florescence emission spectra of the four dyes; the same line types as in *a*, are used to denote the dyes. *c*, Chemical structures of the four dyes. X, The moiety to which the dye is bound, for example, an oligonucleotide primer.. **Methods.** All spectra were obtained in 10 mM sodium carbonate buffer, *p*H 9.0; absorption spectra were taken on an H/P 8451 spectrophotometer; fluorescence spectra were taken on a Perkin-Elmer MPF4 spectrofluorimeter (uncorrected). The following dye derivatives were used for measurements: fluorescein isothiocyanate (FITC), NBD aminohexanoic acid, Texas Red (all from Molecular

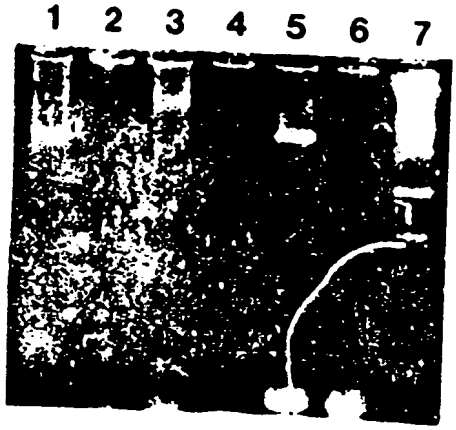
FIG 2

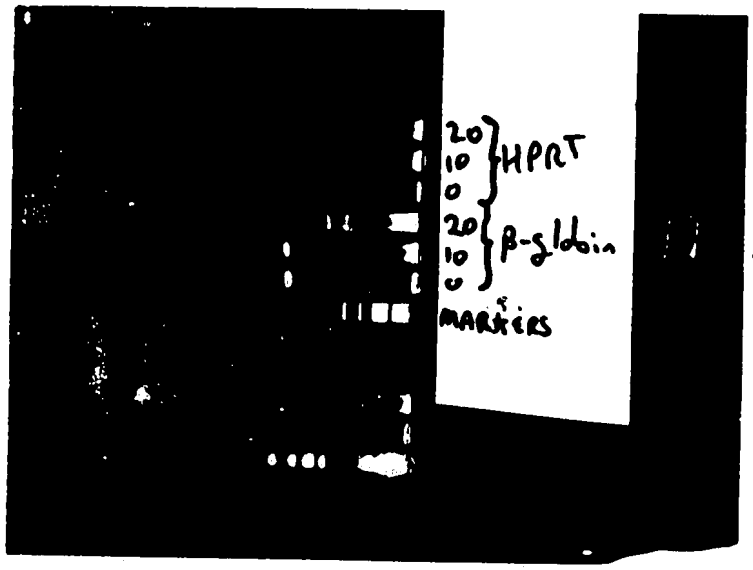




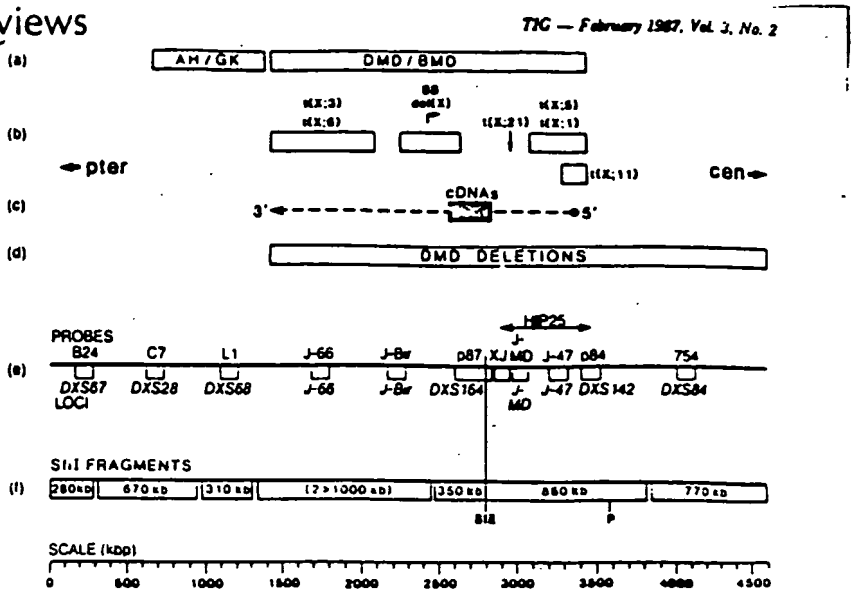

CCCCCCCC
CCCCCCCC
GGGGGGGG

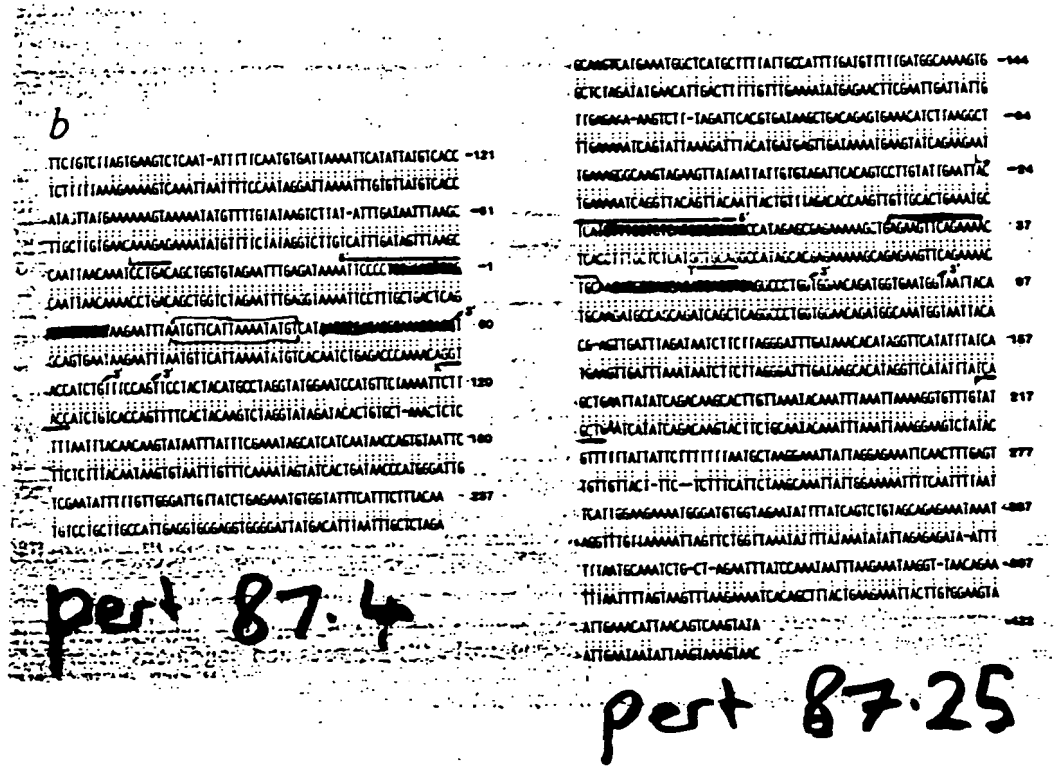

HPRT mRNA
cDNA
AUG
XhoI
Hind III
UAA
1
2
3
4
5
6
basepairs
0
250
500
750


1 2 3 4 5 6 7


20
10
0
HPRT
20
10
0
β-globin
MARKERS

reviews



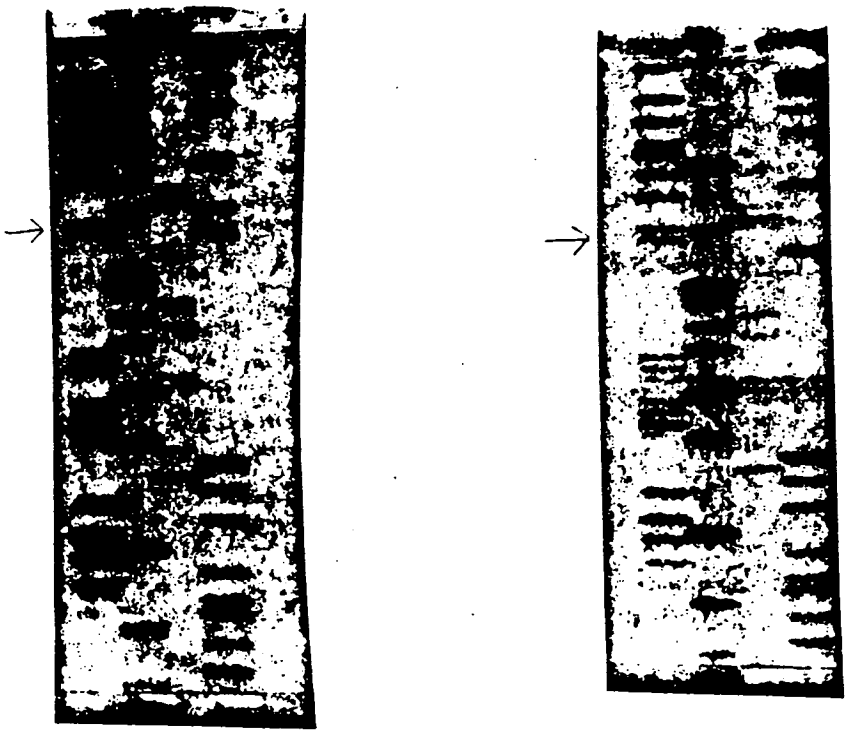


1. PCR Peit
2. PCR HPRT / αIAT / β-globin.
3. NuSieve Gel
4. Probe with internal oligo's. (gel or dot blot)

DNA sequencing ladders of wild type (+) and sparse fur (spf) OTC cDNA constructs, showing the spf mutation; a C to A transverson at position 348. The templates were 'full length' OTC cDNA's, cloned into the eukaryotic expression vector p91023B. Sequencing was by the method of Chen and Seeburg (1985, DNA 4, 165) using double stranded DNA as a template. The same oligonucleotide primer used in the PCR reaction to isolate the spf mutation was employed for the sequencing of this construct. The DNA strand shown is the - strand, i.e. complementary to the cDNA strand shown in fig. one of the manuscript. The order of the sequencing tracks is T, C, A, G.

+

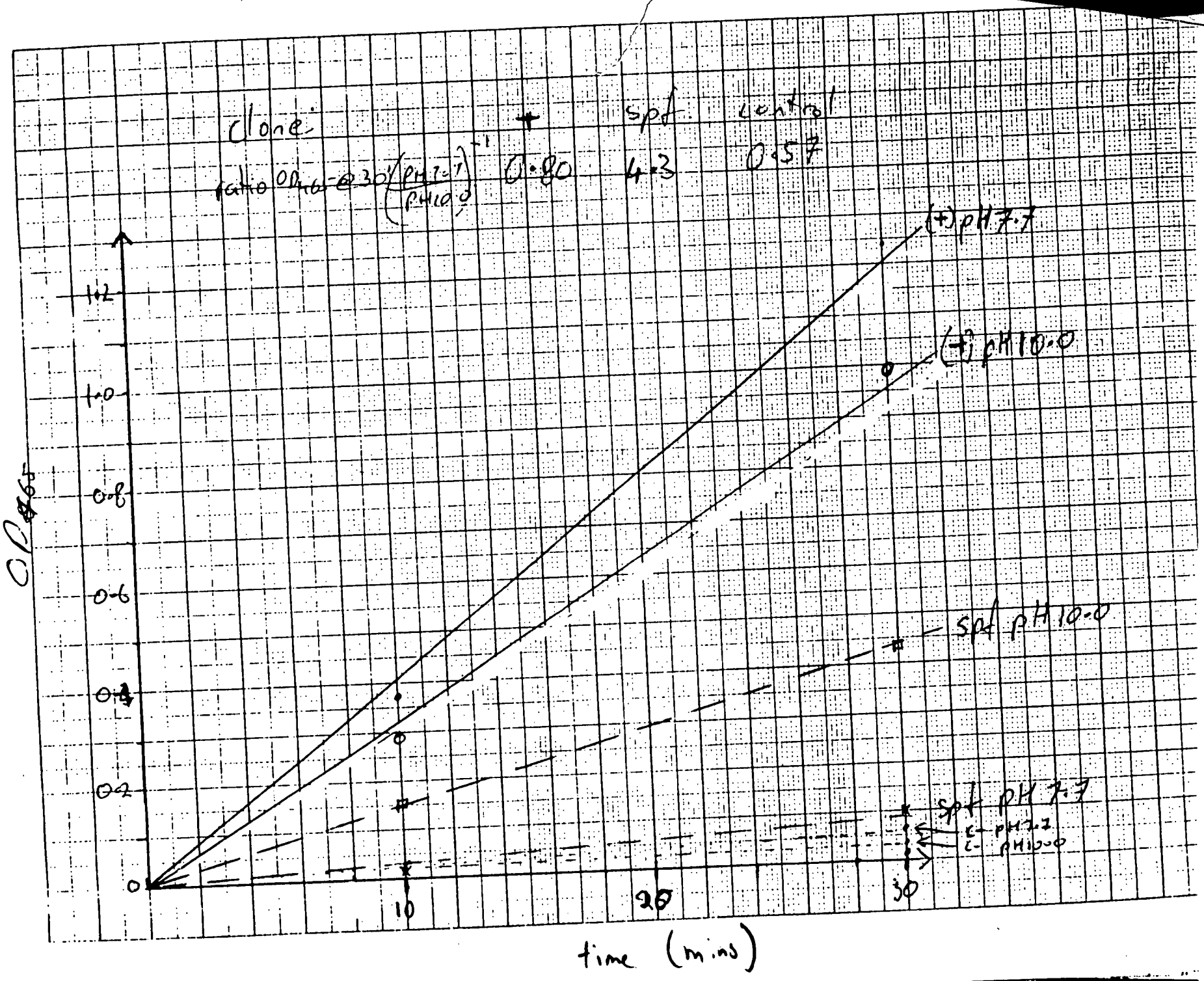
spf



Joel Ravier

EV-[illegible]5'

10	20	30	40	50	60
ACCCAAATAC	TTTGTTCATG	TTTAAATTTT	ACAACATTTC	ATAGACTATT	AAACATGGAA
70	80	90	100	110	120
CATCCTTGTG	GGGACAAGAA	ATCGAATTTG	CTCTTGAAAA	GGTTTCCAAC	TAATTGATTT
130	140	150	160	170	180
GTAGGACATT	ATAACATCCT	CTAGCTGACA	AGCTTACAAA	AATAAAAACT	GGAGCTAACC
190	200	210	220	230	240
GAGAGGGTGC	TTTTTTCCCT	GACACATAAA	AGGTGTCTTT	CTCTCCTTGTA	TCCTTTGGAT
250	260	270	280	290	300
ATGGGCATGT	CAGTTTCATA	GGGAAATTTT	CACATGGAGC	TTTTGTATTT	CTTTCTTTGC
310	320	330	340	350	360
CAGTACAACT	GCATGTGGTA	GCACACTGTT	TAATCTTTTC	TCAAATAAAA	AGACATGGGG
370	380	390	400	410	420
CTTCATTTTT	GTTTTGCCTT	TTTGGTATCT	TACAGGAACT	CCACGGATGGC	ATTGGGCAGC
430	440	450	460	470	480
GGCAAACTGT	TGTCAGAACA	TTGAATGCAA	CTGGGGAAGA	AATAATTCAG	CAATCCTCAA
490	500	510	520	530	540
AAACAGATGC	CAGTATTCTA	CAGGAAAAAT	TGGGAAGCCT	GAATCTGCGG	TGGCAGGACG
550	560	570	580	590	600
TCTGCAAACA	GCTCTCAGAC	AGAAAAAAGA	GGTAGGGCGA	CAGATCTAAT	AGGAATGAAA
610	620				
ACATTTTAGC	AGACTTTTTA	AGCTT			



[redacted]/7 Titering the Human Genomic Library (David Nelson)

- titer thought to be 2×10^{10}
- want 50-100 plaques
- 50 → 1×10^3/ml
  but titer thought to be 2×10^{10}
  50 → 2×10^3/ml

10^{10}
↓ 1/100
10^8
↓ 1/100
10^6
↓ 1/100
10^4
↓ 1/10
10^3

⇒ $1/10^7$ dilution

(50 x of $1/10^7$) (½ of what expected, 2×10^{10})

$46 \times 20 = 9.20 \times 10^2$/ml $\times 10^7 = 9.2 \times 10^9$/ml

use $1/10^4$ dilution [titer 9.2×10^5]

$5 * 10^4 \div 9.2 \times 10^5 = .054 = 54.3\,\mu l$

$4 \times 10^4 \div$ " $= .0435 = 43.5\,\mu l$

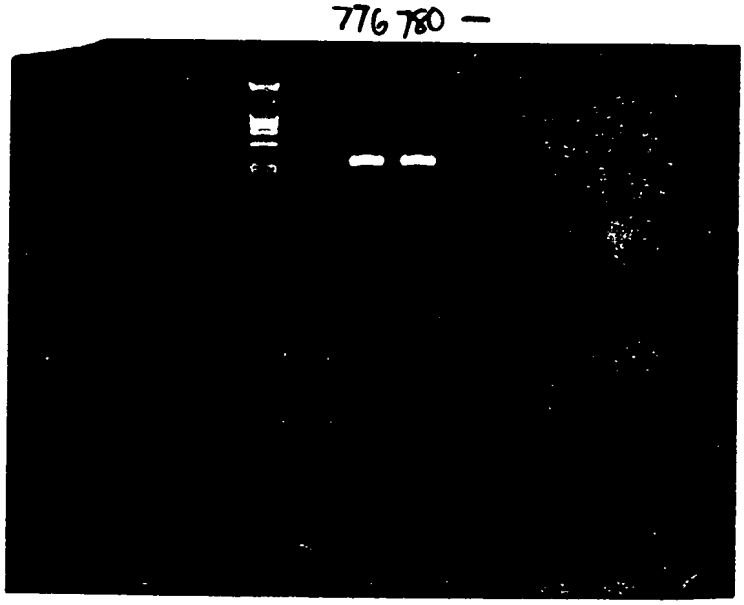
X take $1/10^2$ take 20 into 2ml

used all of $1/10^4$ dilution $\cong 990\,\mu l$

Joel Ranier

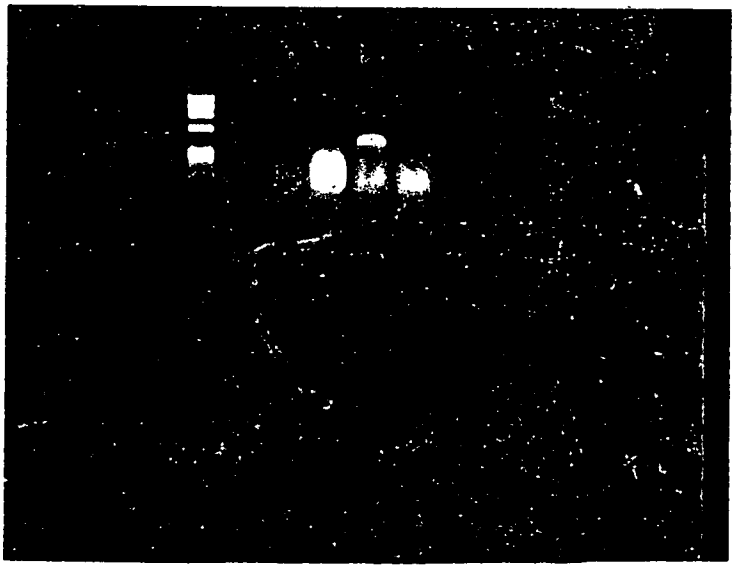
PCR to Test Oligo's 251, 252 (#1) 3/4/8

	1	2	3	(+) control	(+) control	6	(−) control	8	9
1	template			776	no template		780		
2				0.5 µl			0.8 µl		
3	primer 251			0.5	0.5		0.5		
4	(198 µM)			(1 µM)					
5	primer 252			0.8	0.8		0.8		
6	(129 µM)			(µM)					
7									
8	5x Taq buff			20	20		20		
9									
10	dNTP			6	6		6		
11									
12	dH_2O			62.2	62.7		61.9		
13									
14	DMSO			10	10		10		
15				100					
16									
17	94°C	7' cfg	3 sec,	.5 Taq					
18	37°C	30 sec							
19	65°	2'							
20									
21	60 mJ	1% TBE							
22									
23	94°C	1'							
24	37°C	30"							
25	65°	1'							
26									
27										↓									
28					↓									↓				
29															
30									
31									



PCR (second) C primers 251, 252) to amp Mandel exon 3/7/88

	1	2	3	(+) control	(+) control	(-) control no template	(-) control	8	9
1	Template			776	776		158	(deleted)	
2				0.5 λ	0.5 λ	—	1 λ	0.6 μg/ml	
3	primer 251			0.5 λ	0.5 λ	0.5 λ	0.5 λ		
4	(198 μM)			(1 μM)					
5	primer 252			0.8 λ	0.8 λ	0.8 λ	0.8 λ		
6	(129 μM)			(1 μM)					
7	primer 221			1.1 λ	—	—	— λ		
8	(94.4 μM)			(1.0 μM)					
9	primer 222			0.8 λ	—	—	—		
10	(121.7 μM)			(1.0 μM)					
11	5x Taq			20	20	20	20		
12									
13	dNTP			6	6	6	6		
14	25mM								
15	dH_2O			52.8	62.2	62.7	61.7		
16									
17	DMSO			10	10	10	10		
18				100 λ					
19	94° 7' cfg 3sec								
20	.5 λ Taq			loaded 776 (1 set), 776 (2 sets), 158 del., no temp.					
21	37° 30sec								
22	65° 2'								
23									
24	94 1'								
25	37° 30sec								
26	65° 1'								
27									
28																	
29																	
30														
31														



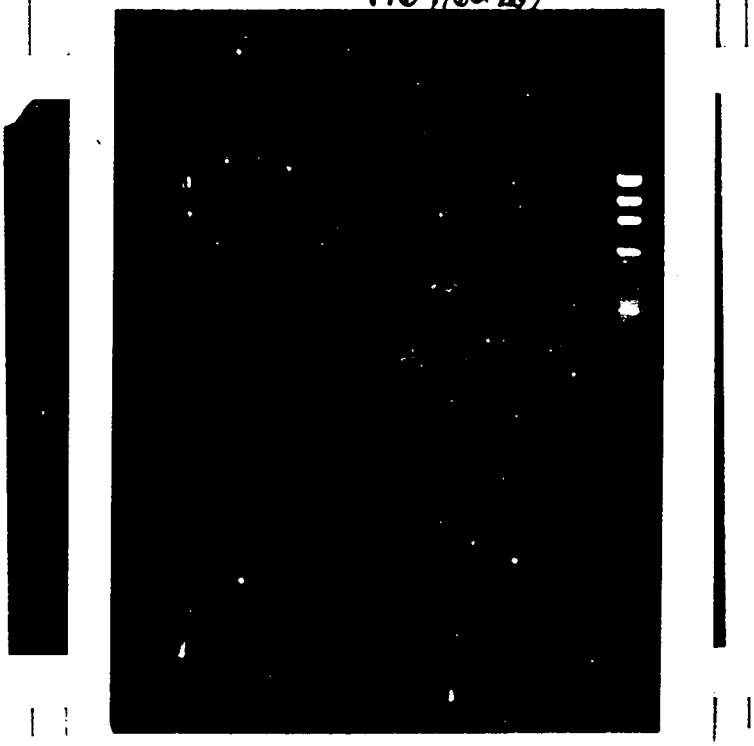
EFFICIENCY LINE® 22-206 AMPAD

PCR #3

3/9/8

	1	2	3 (+) control	4 (+) control	5 (-) control	6 (-) control	7 (-) control	8	9
1	template		776	776	641	665	—		
2			0.5λ	0.5λ	0.25λ	2.5λ	—		
3	primer	251	0.5λ	0.5λ	0.5λ	0.5λ	0.5λ		
4			(1μM)						
5	primer	252	0.8λ	0.8λ	0.8λ	0.8λ	0.8λ		
6									
7	primer	221	1.1λ	—	—	—	—		
8									
9	primer	222	0.85λ	—	—	—	—		
10									
11	5x Taq		20	20	20	20	20		
12									
13	dNTP		6	6	6	6	6		
14									
15	dH_2O		60.2	62.2	62.4	60.2	62.1		
16									
17	DMSO		10	10	10	10	10		
18									
19	776	1μg/ml							
20	641	2.5μg/ml							
21	665	.2μg/ml							
22									
23	94°C 1'	45, 3sec							
24	37°C 30s								
25	65°C 2'								
26									
27	(diff. enz, nucs)								
28	IIII III	94°C 1'							
29	IIII III	37°C 30s							
30	IIII III	65°C 1'							
31	IIII IIII								

set
776 776 641 665 –



AMPAD EFFICIENCY LINE® 22-206

PCR #4 (To test double primer set) 3/10/8

EFFICIENCY LINE™ 22-206 AMPAD

Mix for 3 rxn's (w/o) oligo's

	For 1 RXN	For 3 RXN's
template (776)	4λ	12λ
5x Taq	20λ	60λ
dNTP	6λ	18λ
dH_2O	~~56.80λ~~	170.3λ
DMSO	10λ	30λ

Add 96.77 to 3 tubes

	776 (2 sets)	776 (1st set)	776 (2nd set)
H_2O	—	2.0λ	1.3λ
pr. 25-1	0.5λ	0.5λ	—
pr. 25-2	0.8λ	0.8λ	—
pr. 22-3	1.1λ	—	1.1λ
pr. 22-2	0.83λ	—	0.83λ

No Amplification

Joel Rainer

PCR #5 (c̄ new enzyme) 3/17/8

Mix for 3 rxns (w/o oligo's)

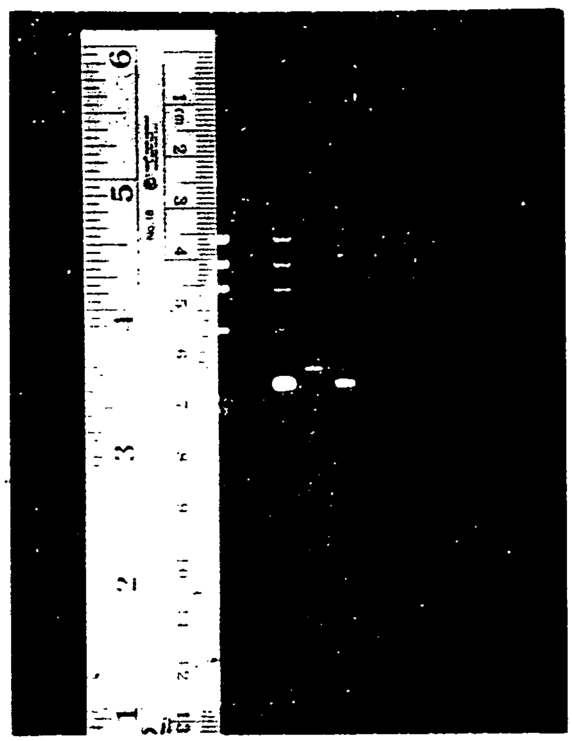
	For 1 RXN	For 3	For no template
template (776 dil)	4		
5x Taq	20		
dNTPs	6		
dH_2O	56.8		
DMSO	10		

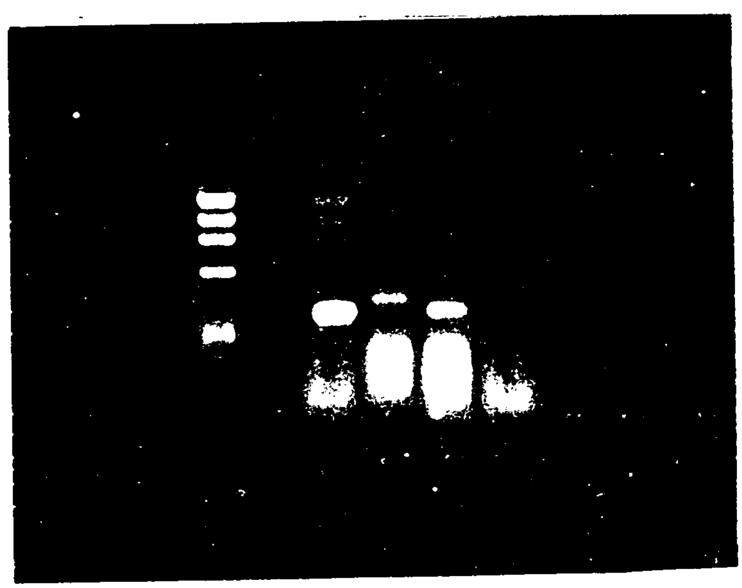
Add 96.8 to each tube

	776 (2 sets)	776 (1st set)	776
nr. 251	0.5	0.5	
252	0.8	0.8	
221	1.1	—	
222	0.8	—	
H_2O	—	2.0	

94°C 1' cfg 3sec
37°C 30sec
Add enz.
65° 2'
94°C 1'
37°C 30sec
65°C 1'30"

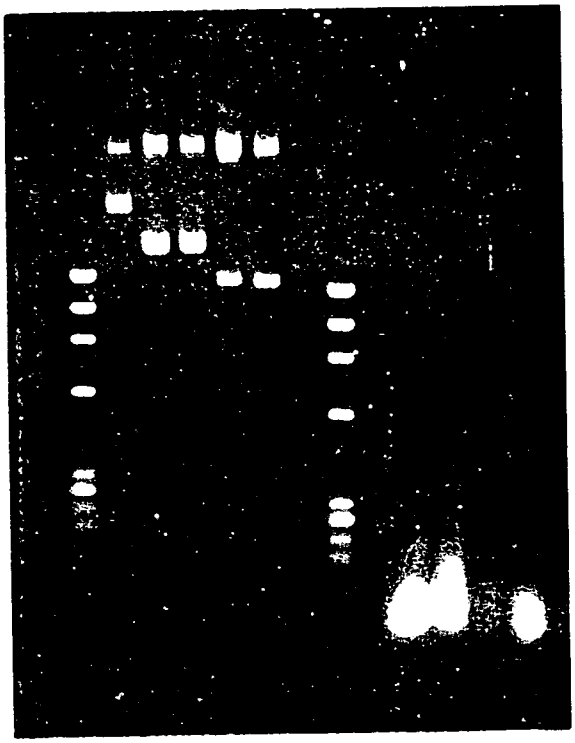
776 1st 2nd 1st set blk ✓

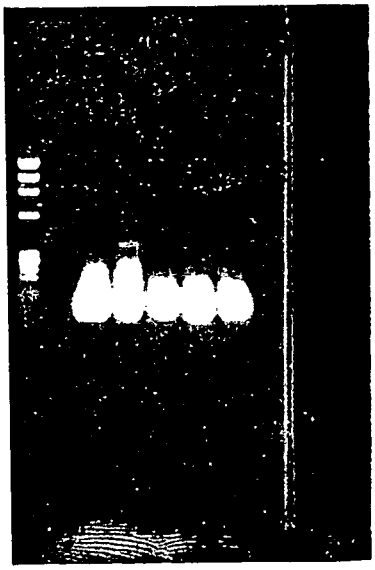




PCR #6 (to test variables) 3/21/8

	1	2	3	4 776	5 776	6 776	7 776	8 776	9
1	template			4	4	4	4	4	
2	(776)								
3									
4	Primer	221		1.6	1.1	1.1	1.1	11	
5		222		~~0.8~~ 1.1	0.8	0.8	0.8	0.8	
6		251		0.25	0.5	0.5	0.5	0.5	
7		252		0.40	0.8	0.8	0.8	0.8	
8									
9	dNTP's			6	6	12	6	6	
10									
11	5x Taq			20	20	20	20	20	
12									
13	DMSO			10	10	10	10	10	
14									
15	H_2O			56.65	56.8	50.8	56.8	56.8	
16						100 λ			
17	Temp. Variable								
18									
19	④ Annea								
20	⑤ "								
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

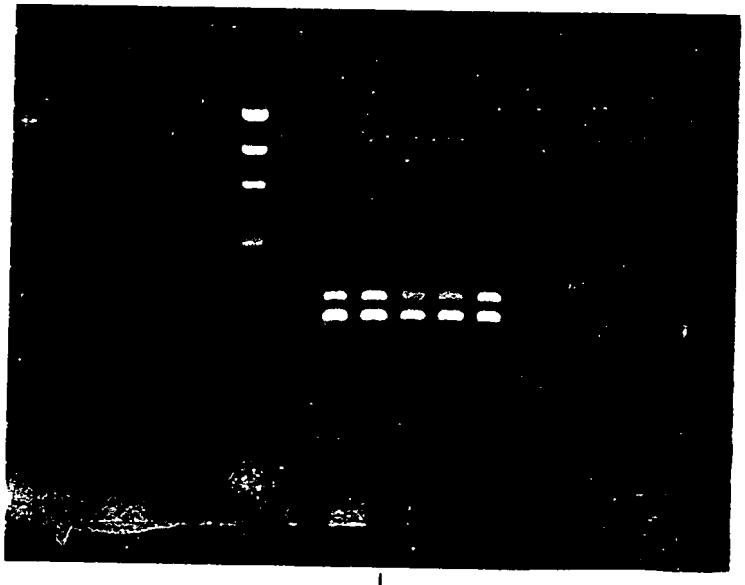




Joel Ramirez

PCR #7 (to test new nuc's, enz, stringency) 3/23/8

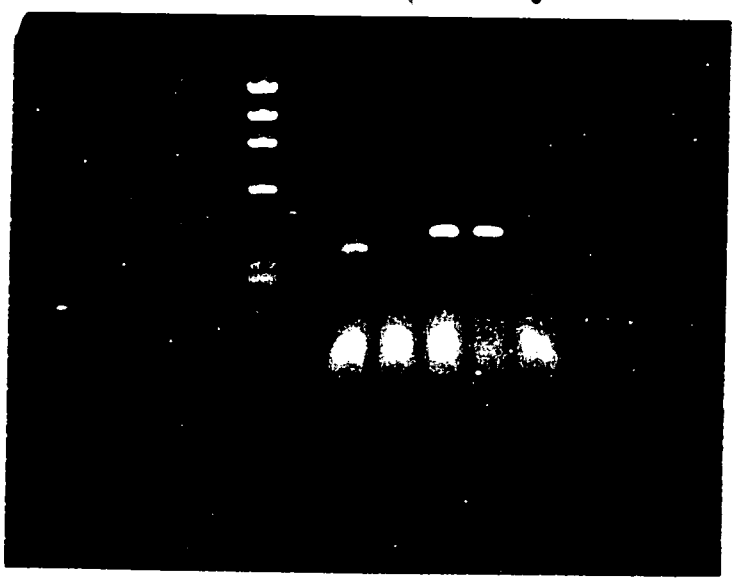
	1	2	3	4	5	6 √2-7	7	8	9
1					776	776	776	776	776
2	template				4	4	4	4	4
3									
4	primer	221	(48 μM)		2	2	2	2	2
5		222	(56 μM)		1.8	1.8	1.8	1.8	1.8
6		251	(198 μM)		0.5	0.5	0.5	0.5	0.5
7		252	(129 μM)		0.8	0.8	0.8	0.8	0.8
8									
9	dNTP's				6 old	6 new	6 old	6 new	6 new
10					(old enz)	(new enz)	(new enz)	(old enz)	(new enz)
11									
12	5X Taq				20	20	20	20	20
13									
14	DMSO				10	10	10	10	10
15									
16	H_2O				54.9	54.9	54.9	54.9	54.9
17									
18									450
19									
20									
21									
22									
23									
24									
25									
26									
27									
28	//// ///								
29	//// ///								
30	//// ///								
31	//// ///								



TOPS FORM 3619

PCR #8 (To test diagnostic cases) 3/24/8

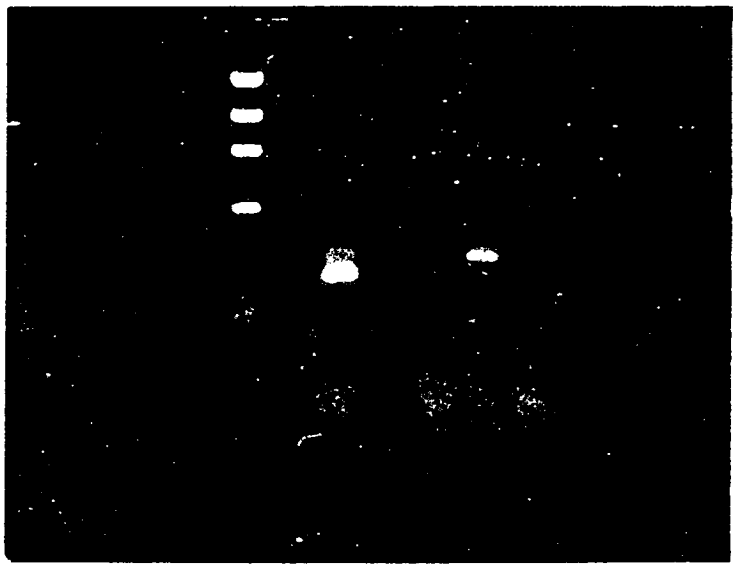
	1	2	3	4	5 776	6 JB	7 665	8 680	9 —
1					normal	double deletion	42 -75, +87	Haddicon (475-87)	no
2	template				4	1.67	2.5	0.66	template
3					(.125 µg/µl)	(0.3 µg/µl)	(0.2 µg/µl)	(0.76 µg/µl)	
4	primer	221	(48 µM)		2	2	2	2	2
5		222	(56 µM)		1.8	1.8	1.8	1.8	1.8
6		251	(198 µM)		0.5	0.5	0.5	0.5	0.5
7		252	(129 µM)		0.8	0.8	0.8	0.8	0.8
8									
9	dNTP's				6	6	6	6	6
10									
11	5x Taq				20	20	20	20	20
12									
13	DMSO				10	10	10	10	10
14									
15	H_2O				54.9	57.2	56.4	58.24	58.9
16							100 µl		
17	94°C	7'	cfg						
18	37°C	30"							
19	.5 x Taq Pol						√	√	
20									
21									
22									
23									
24									
25									
26	Annealed at 40°C								
27									
28	𝍸 𝍸								
29	𝍸 𝍸								
30	𝍸 𝍸								
31	𝍸 ||||								



AMRAD EFFICIENCY LINE - 22-206

PCR #9 (to test 3rd set oligo's + diagnostic 3/27/8

	1	2	3	4	5 (use)	6	7	8	9
1				4λ	4λ	4λ	2.5λ	0.7λ	
2	template			776 (2 sets)	776 (new set)	776 (all sets)	665 (2 sets) -75, +87	660 (2 sets) +87, -87	
3					—				
4	primer	221	(48 μM)	2		2	2	2	
5		222	(56 μM)	1.8		1.8	1.8	1.8	
6		251	(198 μM)	0.5		0.5	0.5	0.5	
7		252	(129 μM)	0.8		0.8	0.8	0.8	
8		276	(106 μM)	—	1.0	1.0	—	—	
9		277	(143 μM)	—	0.7	0.7	—	—	
10									
11	5x Taq			20	20	20	20	20	
12									
13	dNTP's			6	6	6	6	6	
14									
15	DMSO			10	10	10	10	10	
16									
17	H_2O			54.9	58.3	53.2	56.4	58.2	
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28											
29											
30											
31											



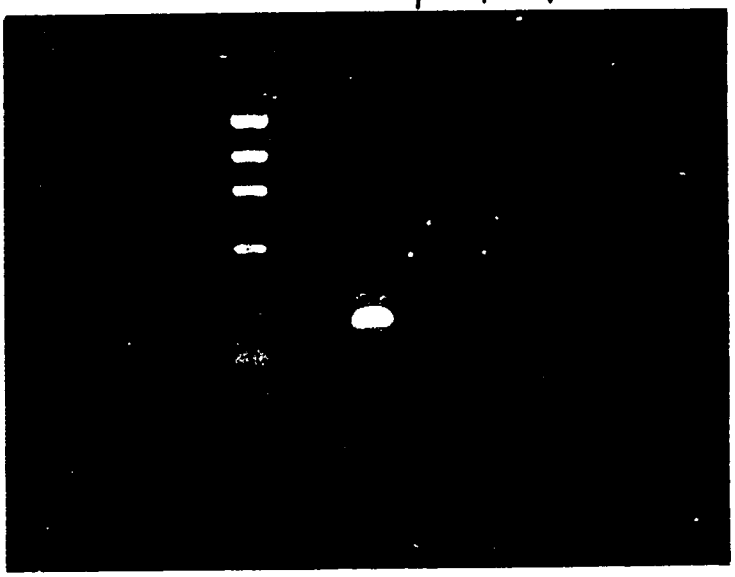
EFFICIENCY LINE 22-206 AMPAD

PCR #10 #4 (To test new oligo's from 44.1) 3/28/7

	1	2	3	4	5776	6776	—	8	9
1									
2	template				4	4	—		
3									
4	primers	221			2	—	—		
5		222			1.8	—	—		
6		251			0.5	—	—		
7		252			0.8	—	—		
8		776				1.0	1.0		
9		277				0.7	0.7		
10									
11	5x Taq				20	20	20		
12									
13	dNTP's				6	6	6		
14									
15	DMSO				10	10	10		
16									
17	H2O				54.9	58.3	62.3		
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

EFFICIENCY LINE #22-206 AMPAD

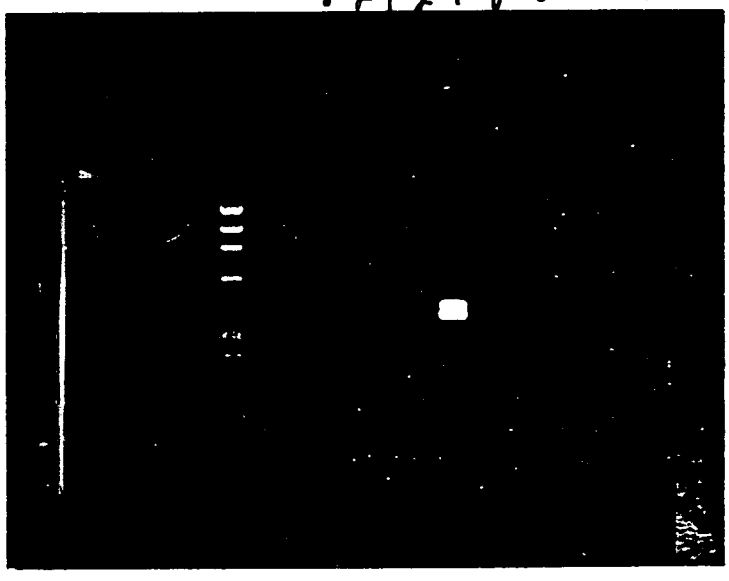
2 old sets | new set | no template



Joel Ranier

PCR #12 (To test assay conditions) 3/30/8

	1	2	3	4	5	6	7	8	9
1					776	776			
2	template				6	6	—		
3									
4	primers	221			2	2	~~2~~		
5		222			1.8	1.8	~~1.8~~		
6		251	(24μM)		2	~~4~~ 2	4		
7		252	(30μM)		1.7	~~3.4~~	3.4		
8									
9	5x Taq				20	20	20		
10									
11	dNTP's				6	6+6 (20)	6		
12									
13	DMSO				10	10	10		
14									
15	H_2O				50.5	46.8	~~48~~ 8		
16									
17	2' extension								
18									
19	Added 4.8λ nuc's at								
29									
30									
31									



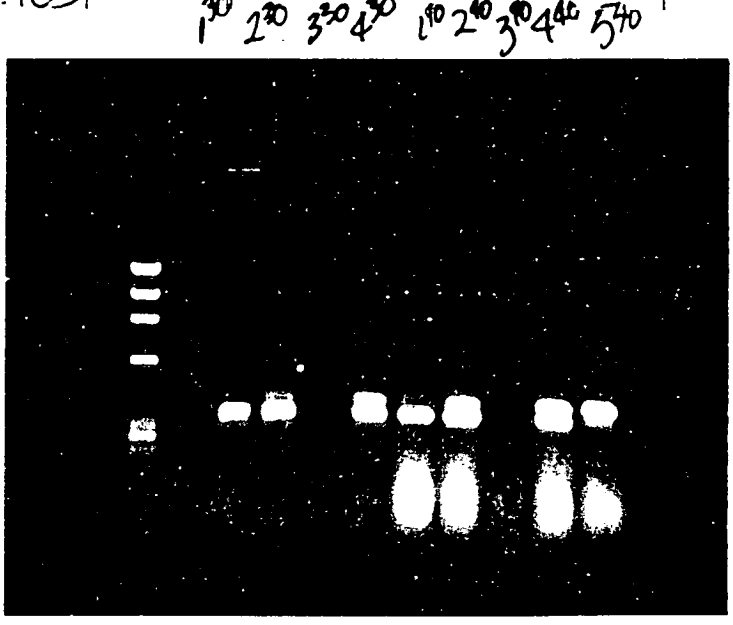
Joel Ramos

PCR #13

3/31/8

	1	2	3	4 1	5 2	6 3	7 4	8 5	9
1				776	776	776	776		
2	template			6	6	6	6	—	
3									
4	pr.	221		2	2	2	2	2	
5		222		1.8	1.8	1.8	1.8	1.8	
6		251		4	4	4	2	—	
7		252		3.4	3.4	3.4	1.7	—	
8									
9	5x Taq			20	20	20	20	20	
10									
11	dNTP's			6	6	6+6	6	6	
12									
13	DMSO			10	10	10	10	10	
14									
15	H_2O			46.8	46.8	46.8	50.5	60.2	
16									

17 Take out 10λ after 30rds.
18 Add 50λ Paraffin oil to 2
19 Add 6λ nuc's 20rds. to 3
20 2' extension
21
22 Added DNA to 5?
23
24
25
26
27
28
29 Nucs →
30
31



Joel Ranier

PCR #14 (to make sure no contamination) 4/4/87

	1	2	3	4	5 776	6 776	7 776	8	9
1					6	—	—		
2	template								
3									
4	primers		221		2	2			
5			222		1.8	1.8			
6			251		2		2		
7			252		1.7		1.7		
8									
9	5xTaq				20	20	20		
10									
11	dNTP's				6	6	6		
12									
13	DMSO (fresh)				10	10	10		
14									
15									
16	H_2O				50.5	60.2	60.4		
17									
18	2' extension								
19	30 rounds								
20	1λ Taq Pol								
21									
22									
23									
24									
25									
26									
27									
28	//// ////								
29	//// ////								
30	//// ////								
31									

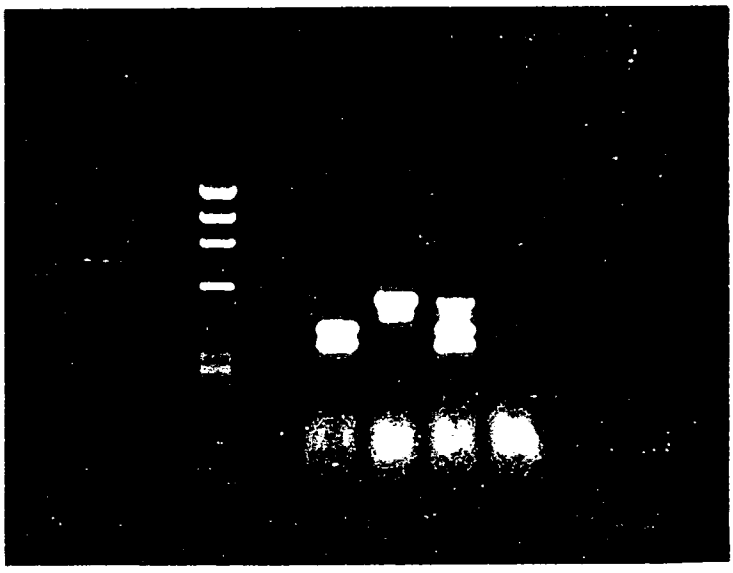
EFFICIENCY LINE 22-206

Joel Ravier

PCR #15 (to test 3rd set)

4/5/8

	1	2	3	4 1	5 2	6 3	7 4	8	9
1				776	776	776	—		
2	template			6	6	6			
3									
4									
5	primers	221	1 μM	2		2	2		
6		222		1.8		1.8	1.8		
7		251	0.5 μM	2		2	—		
8		252	"	1.7		1.7	—		
9		276	1 μM	—	1.0	1.0	—		
10	(44.5 μM)	303		—	2.25	2.25	—		
11									
12	5x Taq			20	20	20	20		
13									
14	dNTP's			6	6	6	6		
15									
16	DMSO			10	10	10	10		
17									
18	H_2O			50.5	~~54.15~~	47.25	~~60~~ 60.2		
19						(# 2 sets) × (new set) (all three) −			
20	2' exten.					1 2 3 4			
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

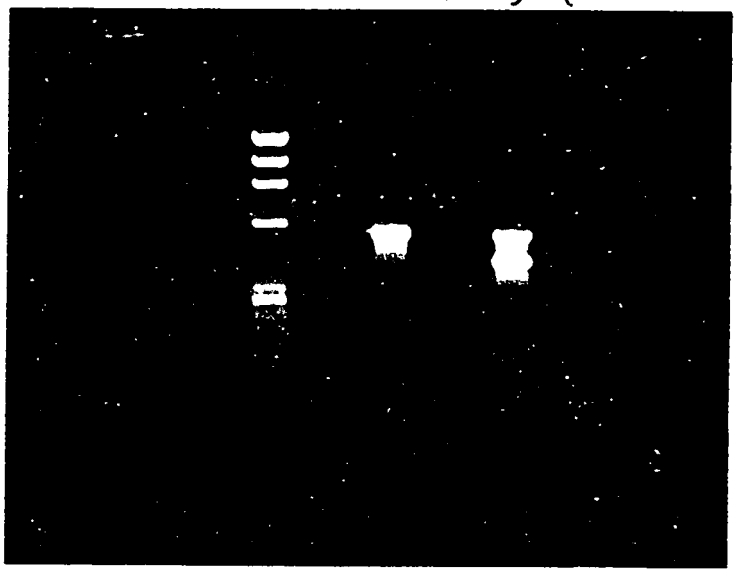


EFFICIENCY LINE® 22-206 AMPAD

Joel Ranier

PCR #16 (to adjust stringency) 4/5/18

	1	2	3	4 1	5 2	6 3	7 4	8	9
1				776	776	776	776		
2	template			6	6	6	6		
3									
4	primers	221		—	—	2	2		
5		222		—	—	1.8	1.8		
6		251		—	—	2	2		
7		252		—	—	1.7	1.7		
8		276		1.0	1.0	1.0	1.0		
9		303		2.25	2.25	2.25	2.25		
10									
11	5x Taq			20	20	20	20		
12									
13	dNTP's			6	6	6	6		
14									
15	DMSO			10	10	10	10		
16									
17	H_2O			54.75	54.75	47.25	47.25		
18									
19	2' extension			45°C	55°C	45°C	55°C		
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



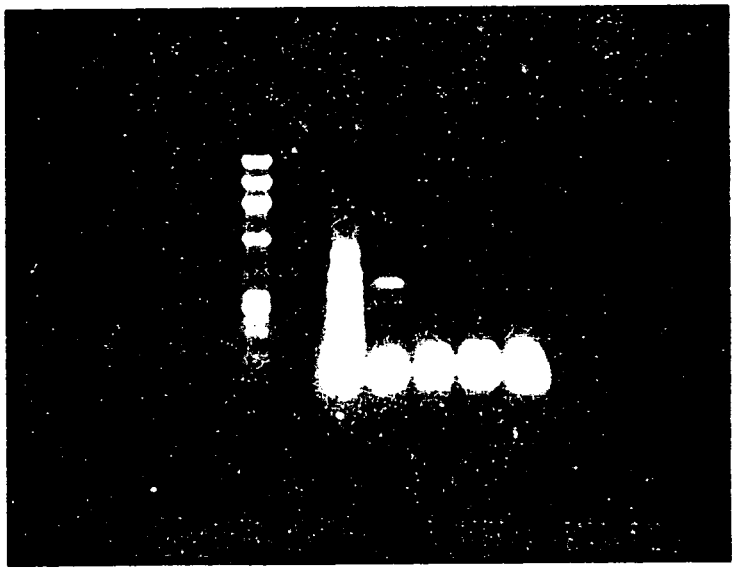
EFFICIENCY LINE™ 22-206 AMPAD

PCR #17 (to adjust clone conc. for 3 sets) 4/7/18

	1	2	3 1	4 2	5 3	6 4	7 5	8 6	9
1			7.6	7.6	7.6	—	—	—	
2	template		6	6	6				
3									
4	primers	221 (#1)	2.2	2.2	1.1	2.2	—	—	
5	(#2)	222	2.4	2.4	1.2	2.4	—	—	
6	(41.8)	251	1.2	1.2	0.6	—	2.4	—	
7	(47.2)	252	1.01	1.01	0.5	—	2.0	—	
8	(45.4)	276	—	4.4	2.2	—	—	2.2	
9	(44.5)	303	—	4.5	2.25	—	—	2.25	
10									
11	5x Taq		20	20	20	20	20	20	
12									
13	dNTPs		6	6	6	6	6	6	
14									
15	DMSO		10	10	10	10	10	10	
16									
17	H_2O		51.2	42.3	50.15	59.4	59.6	59.6	
18									
19	2° extension								
20	~~pa~~ light min. oil								
21	add enz. once								
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

PCR #18 (To correct contam.) 4/9/8

	1	2	3	4 1	5 2	6 3	7 4	8 5	9
1				776	—	—	—	—	
2	template			6					
3									
4	primers	251		1,2	2.4	—	—	—	
5		252		1.1	2.0	—	—	—	
6		221	—	2.2	—	2.2	—	0.5	(not dil.)
7		222		2.4	—	2.4	—	0.6	
8		276		2.2	—	—	2.2	—	
9		303		2.25	—	—	2.25	—	
10									
11	5x Taq			20	20	20	20	20	
12									
13	dNTP's			6	6	6	6	6	
14									
15	DMSO			10	10	10	10	10	
16									
17	H_2O			46.7	45.8	59.4	59.6	62.9	
18									
19	10 rounds								
20	2' extension								
21	Anneal for 2 min								
22	1st 5 rounds								
23									
24									
25									
26									
27									
28									
29									
30									
31									



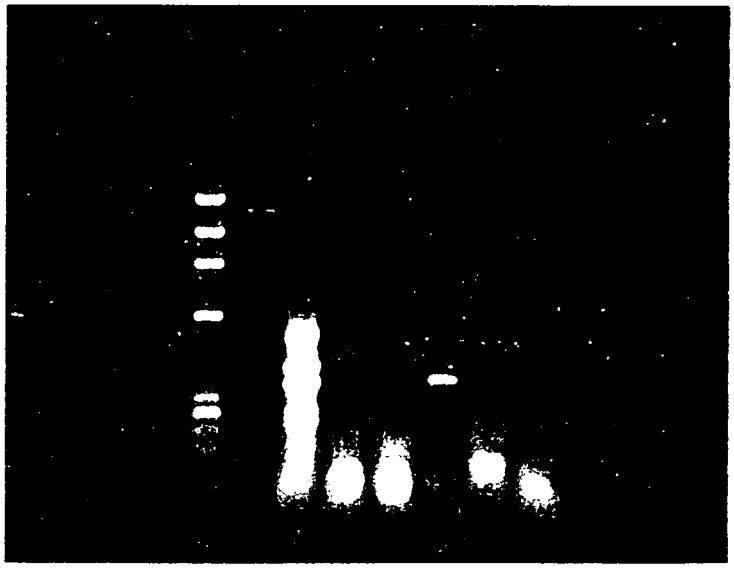
AMPAD EFFICIENCY LINE 22-206

Joel Ranier

PCR #19

4/10/88

	1	2	3	4 1	5 2	6 3	7 4	8 5	9 6
1				776	—	—	—	—	—
2	template			6					
3						(no dil.)			
4	primers	221		2.2	2.2	0.5	—	—	0.5
5		222		2.4	2.4	0.6	—	—	0.6
6		251		1.2	1.2	0.5	0.5	—	—
7		252		1.1	1.1	0.8	0.8	—	—
8		276		2.2	2.2	~~2.2~~ 0.5	—	~~2.2~~ 0.5	—
9		303		2.2	2.2	0.8	—	0.8	—
10									
11	5x taq			20	20	20	20	20	20
12									
13	dNTP's			6	6	6	6	6	6
14									
15	DMSO			10	10	10	10	10	10
16									
17	H_2O			40.7	52.7	~~40.3~~ ~~58.6~~	62.7	62.7 ~~61.0~~	62.9
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



EFFICIENCY LINE™ 22-206 AMPAD

PCR #20

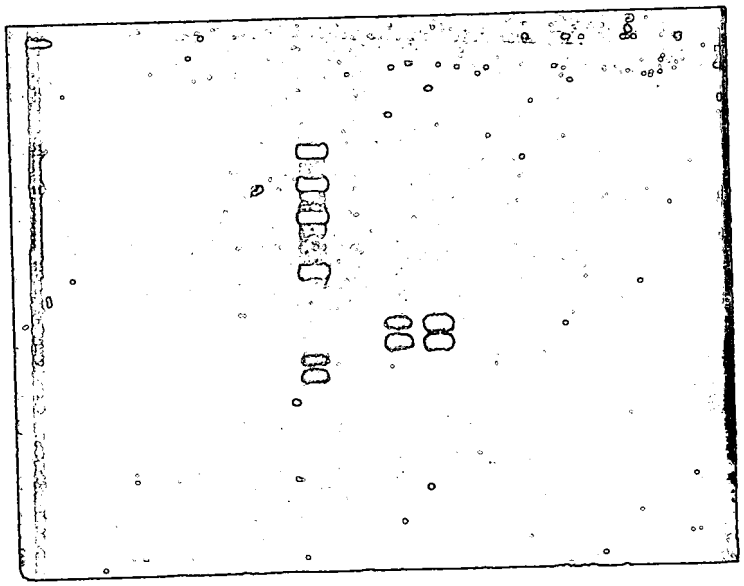
4/14/8

	1	2	3	4 1	5 2	6 3	7	8	9
1				776	776	—			
2	template			4	4				
3									
4									
5	Primers	221	(1 μM)	2.2	2.2	2.2			
6	(42)	222	"	2.4	2.4	2.4			
7	(51.6)	251	"	1.95	1.95	1.95			
8	(208)	252	"	0.5	0.5	0.5			
9	~~(45.4)~~	276	"	2.2	2.2	2.2			
10	~~(44.5)~~	303	"	2.25	2.25	2.25			
11									
12	5x Taq			20	20	20			
13									
14	dNTP's			6	6	6			
15									
16	DMSO			10	10	10			
17									
18	H_2O			46.5	46.5	52.5			
19									
20	use 2-25u Taq Pol			40°C	43°C	40°C			
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

EFFICIENCY LINE 22-206 AMPAD

5min 65°C

5min 65°C

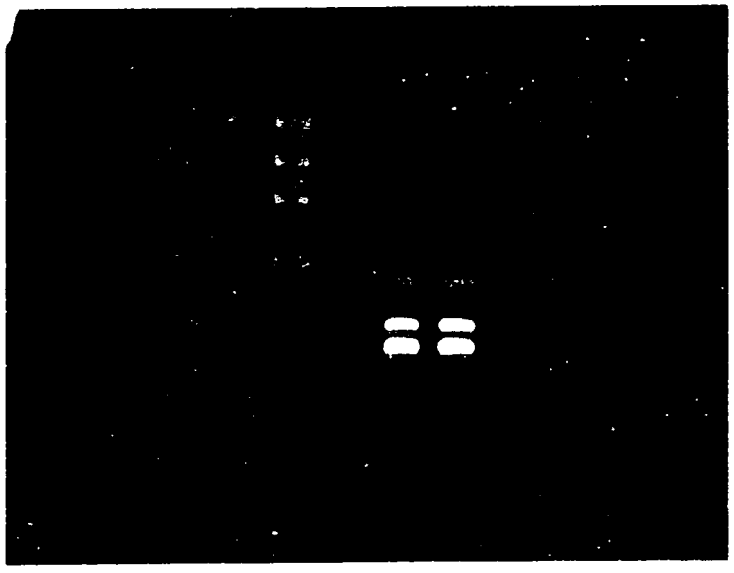


Joel Ramler

PCR #21

4/17/8

	1	2	3	4	5 1	6 2	7 3	8	9
1					776	776	—		
2	template				6	6			
3									
4	primers	221			2.2	2.2	2.2		
5		222			2.4	2.4	2.4		
6		251			1.95	1.95	1.95		
7		252			0.5	0.5	0.5		
8		276			2.2	2.2	2.2		
9		303			2.25	2.25	2.25		
10									
11	5x Taq				20	20	20		
12									
13	dNTP's				6	6	6		
14									
15	DMSO				10	10	10		
16									
17	H_2O				46.5	46.5	52.5		
18									
19	Used excess enz.				37°C	43°C	37°C		
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30	5 min @ 68								
31		extend 5 min							



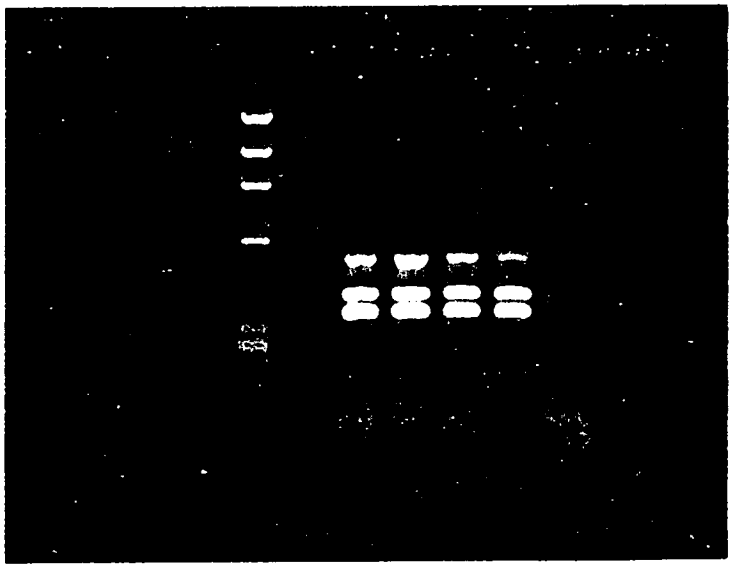
EFFICIENCY LINE 22-206 AMPAD

Joel Ranier

PCR #22

4/18/8

	1	2	3	4 1	5 2	6 3	7 4	8 5	9
1				776	776	776	776		
2	template			6	6	6	6		
3									
4	primers	221		2.2	2.2	2.2	4.4	2.2	
5		222		2.4	2.4	2.4	4.8	2.4	
6		251		1.95	1.95	1.0	1.0	1.95	
7		252		2.2	2.2	1.1	1.1	2.2	
8		276		2.2	2.2	1.1	1.1	2.2	
9		303		2.20	2.20	1.1	1.1	2.2	
10									
11	5x Taq			20	20	20	20	20	
12									
13	dNTP's			6	6	6	6	6	
14									
15	DMSO			10	10	10	10	10	
16									
17	H_2O			74.8	44.8	49.1	44.5	50.8	
18									
19	Add 2λ Taq Pol (5x)								
20	Add 1λ Taq Pol								
21	after 20 rds. to 2								
22									
23									
24									
25									
26									
27									
28	//////								
29	5min ////								
30	65° ////								
31	////								



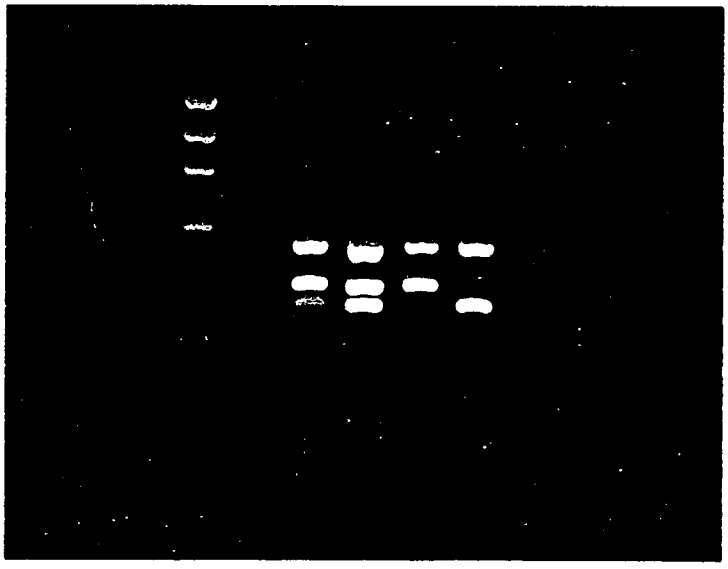
EFFICIENCY LINE® 22-206 AMPAD

Joel Ranier

PCR #23

4/19/88

	1	2	3	4	5 1	6 2	7 3	8 4	9
1					776	776	776	776	
2	template				6	6	6	6	
3									
4	primers	221			1.9	1.9	1.9	—	
5		222			2.0	2.0	2.0	—	
6		251			1.95	1.95	—	1.95	
7		252			2.2	2.2	—	2.2	
8		276			2.2	2.2	2.2	2.2	
9		303			2.2	2.2	2.2	2.2	
10									
11	5x Taq				20	20	20	20	
12									
13	dNTP's				6	6	6	6	
14									
15	DMSO				10	10	10	10	
16									
17	H_2O				45.6	45.6	49.7	49.5	
18									
19	10 u enzyme at start				50°C	43	43	43	
20	Extend 3 min.								
21	Extend 5 min at								
22	20 rounds								
23	Extend 7 min								
24	at end								
25									
26									
27									
28									
29									
30									
31									



EFFICIENCY LINE® 22-206 AMPAD

Joel Ranie

PCR #24 (to test stringency) 4/20/8

			1	2
template			776 6	776 6
primers	221		1.9	1.9
	222		2.0	2.0
	251		1.95	1.95
	252		2.2	2.2
	276		2.2	2.2
	303		2.2	2.2
5xTaq			20	20
dNTP's			6	6
DMSO			10	10
H_2O			45.6	45.6
			100	100

Extend 3.5 min.
10u Taq Pol

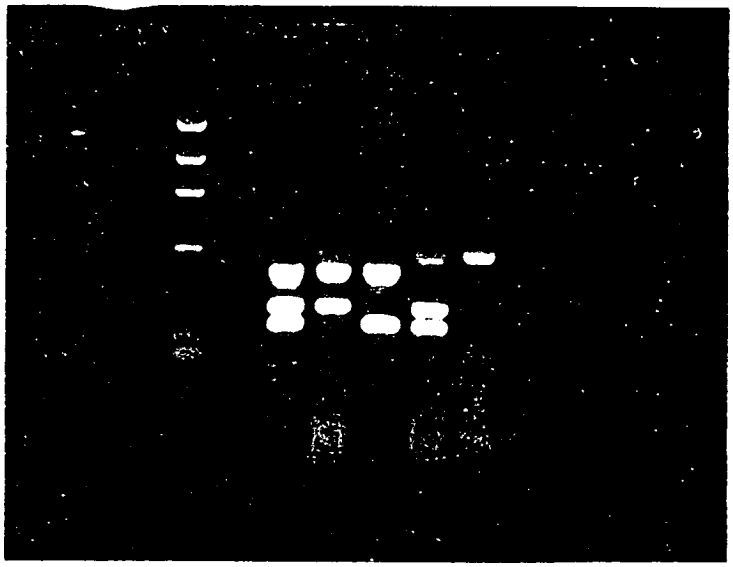
1- 43°C anneal
15sec
2- 46°C anneal
30 sec

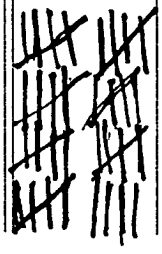
PCR #25

4/20/8

	1	2	3	4 776	5 665	6 660	7 1011	8 JB	9 —
1				1	2	3	4	5	6
2	template			8.6	5.9	9.1	3.5	7.0	—
3									
4	primers		221	1.9	1.9	1.9	1.9	1.9	1.9
5			222	2.0	2.0	20	2.0	20	2.0
6			251	1.95	1.95	1.95	1.95	1.95	1.95
7			252	2.2	2.2	2.2	2.2	2.2	2.2
8			276	2.2	2.2	2.2	2.2	2.5	2.2
9			303	2.2	2.2	2.2	2.2	2.2	2.2
10									
11	5x Taq		20	→					
12									
13	dNTP's		6	→					
14									
15	DMSO		10	→					
16									
17	H_2O			42.0	45.65	42.45	48.1	44.6	51.6
18									

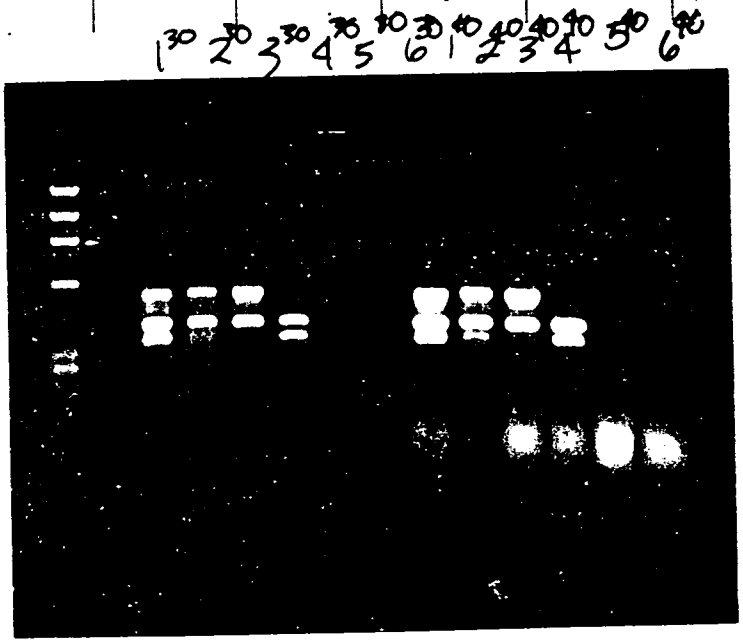
3.5 min extension
46°C anneal 30sec
7 min. denaturation at beginning
7 min extension at end
2x (10u) Taq Pol. at beginning





PCR #26 4/25/8

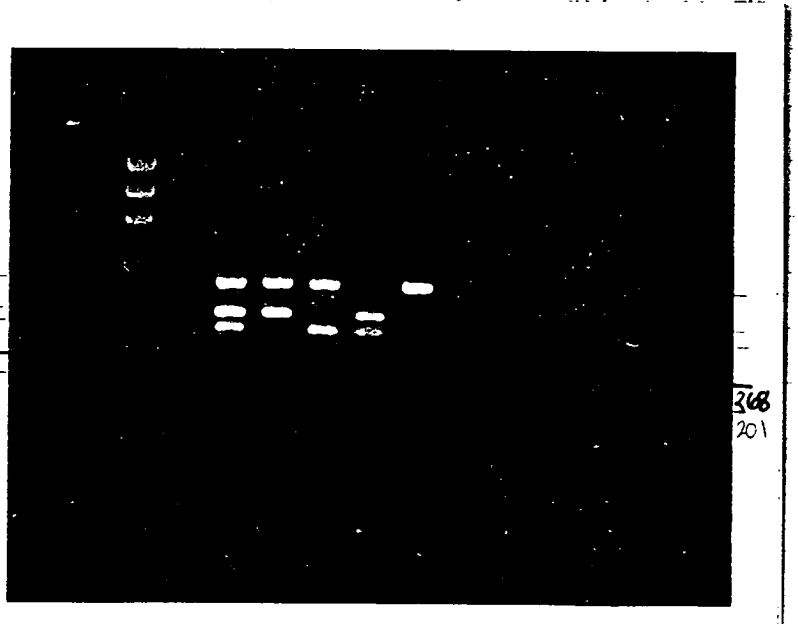
	1	2	3 1	4 2	5 3	6 4	7 5	8 6	9
1			776	776	665	101	IB	—	
2	template		8.6	8.6	5.9	3.5	7.0	—	
3									
4	primers	221	1.9						
5		222	20						
6		251	1.7						
7		252	2.0						
8		276	2.2						
9		303	2.2						
10									
11	5x Taq	20							
12	dNTP's	6							
13	DMSO	10							
14	H_2O		43.4		+2.7	+5.1	+1.6	+8.6	
15	Mix			lenz.					
16	221	11.4							
17	222	12							
18	251	10.2							
19	252	12							
20	276	13.2							
21	303	13.2							
22	5x	120							
23	dNTP's	36							
24	DMSO	60							
25	H_2O	260.4							
26		Add 91.4							
27	47° 3 min. extension								
28									
29									
30									
31									



Joel Ranier

PCR #27 (All deletion combo's) 9/27/8

	1	2	3 1	4 2	5 3	6 4	7 5	8 6	9 7
1			5.56λ	5.9λ	8.7	3.5	5.70	7.0	
2	template		776	665	660	1011	641	JB	—
3			(90ng/λ)	(85ng/λ)	(57.5ng/λ)	(142.5ng/λ)	(87.5ng/λ)	(71ng/λ)	—
4	primers								
5	221	(46μM)	2.17						
6	222	(49μM)	2.0						
7	251	(59μM)	1.7						
8	252	(49μM)	2.0						
9	276	(51μM)	1.96						
10	303	(46μM)	2.17						
11									
12	5x Taq Buff		20						
13	DMSO		10						
14	dNTP's		6						
15			+3.14	+2.8	(43.3)	+5.2	+3	+1.7	+8.7
16	Mix	(7)							
17	221	15.2							
18	222	14	3min extensions						
19	251	11.9	2x Taq Pol.						
20	252	14	30 rounds						
21	276	13.70	47°C						
22	303	15.2							
23	5x	140							
24	DMSO	70							
25	dNTP's	42							
26	H_2O	303							
27	Add	91.3							
28									
29		5x loaded							
30									
31									

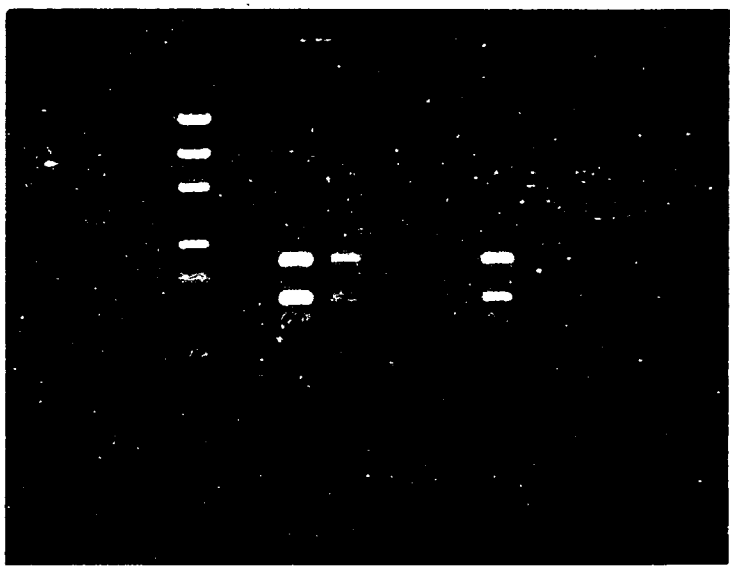


Joel Ranier

PCR (PND's) #28

5/18/8

	1	2	3	4 776	B. Jonon	A. Jonon	7 Posey	Ruggiero	9 —
1				1	2	3	4	5	6
2	template			6	7.5λ	13.3λ	11.9λ	2λ	—
3									
4	primers	221		2.17					
5		222		2.0					
6		251		1.7					
7		252		2.0					
8		276		1.96					
9		303		2.17					
10									
11	5x Taq			20					
12	DMSO			10					
13	dNTP's			6					
14	H_2O			7.3	5.8	—	1.4	11.3	13.3
15									
16									
17	Mix		X6						
18	221	2.17	13.0	2					
19	222	2.0	12.0	4					
20	251	1.7	10.2						
21	252	2.0	12.0						
22	276	1.96	11.76						
23	303	2.17	13.0						
24	5x	20	120						
25	dNTP's	6	36						
26	H_2O	38.7	2322						
27	DMSO	10	60						
28	Add		86.7						
29									
30									
31									



EFFICIENCY LINE™ 22-206

Dilutions for PND's

5/18/8

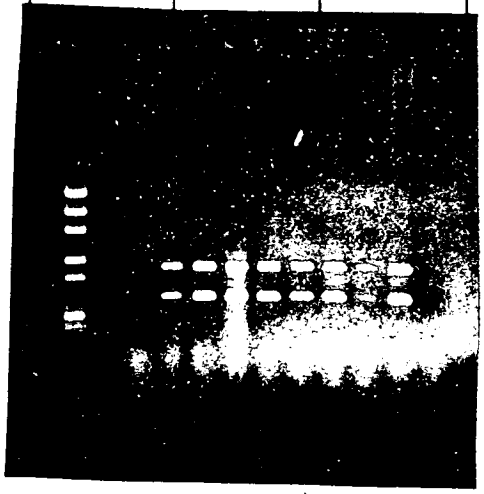
	1	2	3	4	5 DNA	6	7 H_2O	8 A_{260}	9 Conc.
1									
2	Russeu	Jonon	(186ng/λ)		2.3		17.7	.0116	67ng/λ
3	(Amnio)	Jonon	(300ng/λ)		4		16	.009	37.5ng/λ
4	776				8		92	.020	83.3ng/λ
5	Nicholas	Posey	(1.65ng/λ)		1.2		18.8	.185	
6	(Fetus)	Ruggiero	(260ng/λ)		7.7		12.3	.045	188ng/λ
7								0.01	42ng/λ
8								0.060	250ng/λ
9	Nick	Posey						0.058	240ng/λ
10									
11									
12									
13									
14									
15		Branch	3505	1000ng/λ	2		18	0.031	77.5ng/λ
16									
17	Lynn	Rousseau		585ng/λ	3.4		16.6	0.151	378ng/λ
18								0.022	155ng/λ
19	(Fetus)	Rousseau		100ng/λ	—		—		
20									
21	Mary	Richardsen		430ng/λ	4.7		15.3	0.151	378ng/λ
22								0.028	70ng/λ
23	Sharon	Watkins		746ng/λ	2.7		17.3	0.063	200ng/λ
24								0.069	172.5ng/λ
25	Alfred	Watkins		220ng/λ	9.1		10.9		
26									
27	(Fetus)	Watkins		<470ng/λ	4.25		15.75	0.015	37.5ng/λ
28									
29		1504		700ng/λ	2.9		17.1	~~0.192~~	
30								0.199	500ng/λ
31								0.010	25.0ng/λ

Joel Ranier

PCR 29 (more PNDs)

5/18/18

	1	2	3	4	5	6 DNA	7 H_2O	8	9
1									
2	template	(1)	Posey (24ng/λ)			2.08λ	*	off ♂ ×J1.1 → J-Bro	
3		(2)	Branche (77.5ng/λ)			6.5λ	13.5	aff ♂ 9-7	
4		(3)	L. Rousseau (55ng/λ)			9.1λ	10.9	carrier ♀	Bro
5		(4)	F. Rousseau (100ng/λ)			5λ	15	fetus - normal	
6		(5)	M. Richardson (70ng/λ)			7.15λ	12.85	carrier ♀	
7		(6)	S. Watkins (20ng/λ)			2.5λ	17.5	carrier ♀	
8		(7)	A. Watkins (172.5ng/λ)			2.9λ	17.1	aff. male 9-7	Bro
9		(8)	F. Watkins (37.5ng/λ)			13.3λ	6.7	norm. fetus	
10		(9)	1504 (25ng/λ)			20λ	—	aff male 9-7 Bro?	
11		(10)	—					neg. control	
12		(11)	× 1	× 8		* For (1)			
13	primers	251	1.7	13.6		221	2.17		
14		252	2.0	16.0		222	2.0		
15		276	1.96	15.7		251	1.7		
16		303	2.17	17.4		252	2.0		
17		5× Buff	20	160		276	1.96		
18		dNTP's	6	48		303	2.17		
19		DMSO	10	80		Buff	20		
20		H_2O	36.17	289.4		Nucs	6		
21			Add	80		DMSO	10		
22						H_2O	50		
23	2× enz.								
24	47°C anneal								
25									
26									
27									
28									
29									
30									
31									



EFFICIENCY LINE™ 22-206 AMPAD

6/2/88 DNA samples for PCR on DMD males

	DNA #	CONC.	NAME	ORL #
* 1st Priority Group	346 - (1)	.997	George Derry, Jr. - 47-46	22B
	472 (2)	.824	Thomas Davis	70B
	3934 (3)	.599	Shane Worth	521
	3955 (4)	.757	Alan Cox	522
	3920 (5)	.858	Douglas Hazelton - 47-46	523
	3944 (6)	1.054	Billy Buchanan	524
	3948 (7)	.938	Andrew Roybal	531
2nd Priority Group	3929 (8)	.712	Keith young III - 47-46	505
	346		~~Robert Barbee, Jr.~~	~~473~~
	3940 (9)	.220	Scott Miller ?	484
	3860 (10)	1.02	Donald Caputo	513
	3880 (11)	.510	Wayne Noon	514
	3895 (12)	.606	David Van Zandt	519
	3950 (13)	.809	Matthew Stone	520

Joel Ramler

Dilutions for Diagnostic PCR

6/2/18

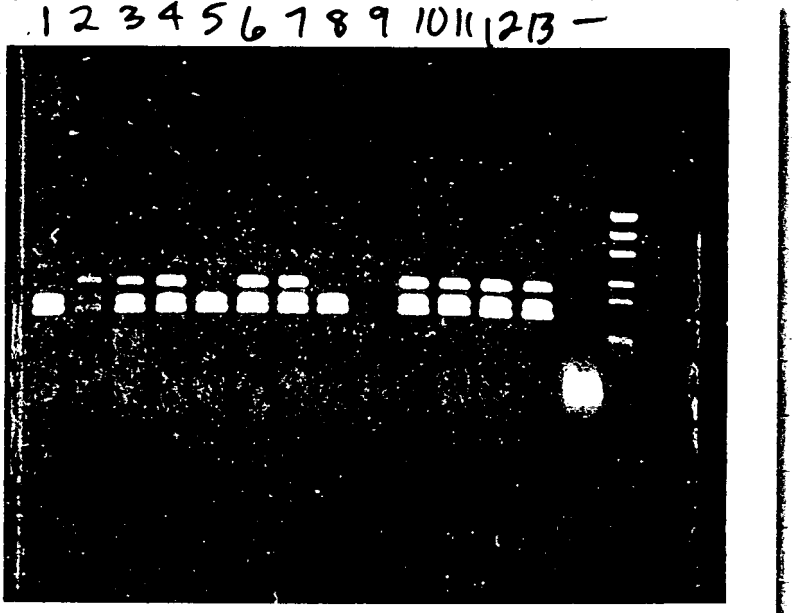
	1 DNA #	2 Conc.	3 For 500ng	4 DNA (µl)	5 H_2O (µl)	6	A_{260}	8 Conc	9 Add H_2O	
1										
2	346	1.0µg/λ	5.23	2.5	17.5		0.181	754ng/λ	+100.69	95.8ng/
3							0.023			
4	472	824ng/λ	1.67	3.0	17.0		0.127	529ng/λ	+64.69	300ng/
5							0.072			
6	3934	600"	8.0	4.2	15.8		0.044	183ng/λ	+9.3	625ng/
7							0.015			
8	3955	757"	7.5	3.3	16.7		0.049	209ng/λ	+12.68	66.7ng/
9							0.016			
10	3920	858"	3.88	2.9	17.1		0.031	129ng/λ		1
11										
12	3944	1.0 µg/λ	5.71	2.5	17.5		0.045	1875ng/λ	+10.00	875ng/
13							0.021			
14	3948	940ng/λ	3.25	2.65	17.35		0.037	154ng/λ		
15										
16	3929	712ng/λ	3.14	3.5	16.5		0.038	158ng/λ		
17										
18	3940	220"	7.5	11.36	8.64		0.131	546ng/λ	+67.36	66.7ng/
19							0.016			
20	3860	1.0µg/λ	8.0	2.5	17.5		0.040	167ng/λ	+6.72	625ng/
21							0.015			
22	3880	510ng/λ	10.0	4.9	15.1		0.012	50ng/λ		
23										
24	3895	606"	4.27	4.13	15.87		0.028	117ng/λ		
25										
26	3950	809	3.76	3.1	16.9		0.032	133ng/λ		
27	Oligo		A_{260}		Conc.					
28	222-1	(27-mer)	.537		150.7µM					
29	221	(27-mer)	0.48		136µM					
30	222-2	(27-mer)	0.73		205µM					
31										

EFFICIENCY LINE™ 22-206
AMPAD

Joel Parker

PCR 30 (diagnostic) 6/4/8

	1	2	3946	9712	3934	3955	3920	3944	3948	3929	3940	3860	3880	3895	3950	—
1			1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	template		5.23	1.67	8.0	7.5	3.88	5.71	3.25	3.16	7.5	8.9	10.9	9.27	3.76	—
3			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
4	H_2O		4.17	8.33	2.0	2.5	6.12	4.29	6.75	6.84	2.5	2.0	—	5.73	6.24	10.0
5																
6	primer	221	(136 μM)													
7		222	(15 μM)													
8		251	(223 μM)													
9		252	(208 μM)													
10		276	(106 μM)													
11		303	(122)													
12																
13	Mix	1x	13x													
14	5x Buff	20	260													
15	DMSO	10	130													
16	dNTPs	6	78 (5)													
17	221	0.74	9.56													
18	222	0.66	8.61													
19	251	0.45	5.83													
20	252	0.48	6.25													
21	276	0.94	12.26													
22	303	0.82	10.66													
23	H_2O	49.9	648.83													
24			Add 90													
25																
26	2x Taq Pol.															
27	47°C															
28																
29																
30																
31																



10λ

EFFICIENCY LINE® 22-206

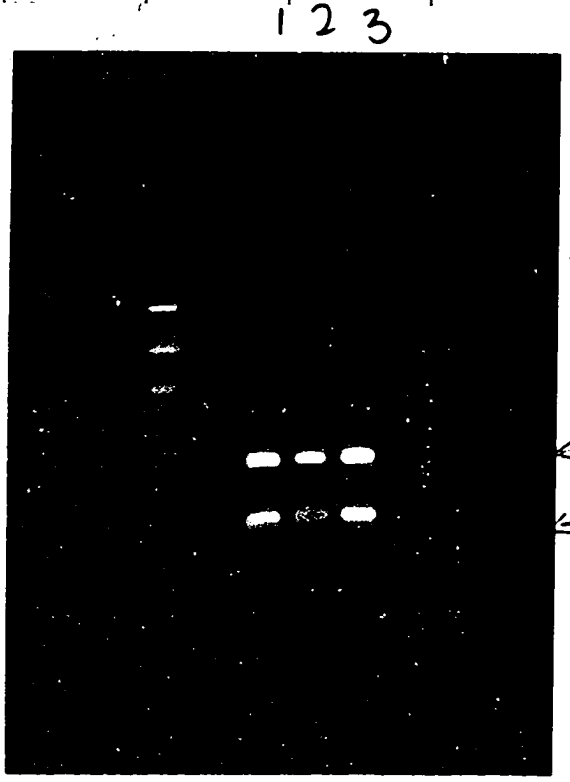
Joel Painter

PCR 31 (diagnostic)

6/9/8

	1	2	3 (1)	4 (2)	5 (3)
1	H_2O	5	3847 5λ	3961 10λ	3968 3λ
2	template	2	5	—	7
3		Mix		1x	4x
4	primers	221	(132μM)	.735	2.94
5		222	(150.2μM)	.66	2.65
6		251		1.7	6.8
7		252		2.0	8.0
8		303	(122μM)	.82	3.28
9		276	(51μM)	1.96	7.84
10	5x Buff	90			
11	DMSO	40			
12	dNTP's	24			
13	H_2O	184.5			
14					
15	Add	90λ			

* Check to see if a primer (221, 222) was left out
see 6/15/8 2,3,4



EFFICIENCY LINE 22-206

Joel Kanter

Dilution of DNA (affected ♂s) 31

6/9/8

	1	2	3	4 DNA	5	6 H_2O	7	8 A260	9 conc
1									
2	3847	782 ng/λ		3.2		16.8		.024	100 ng/λ
3	Douglas Eastman								
4									
5	3961	105 ng/λ		—		—		0.104	
6	Norman Hillman							0.012	50 ng/λ
7									
8	3968	907 ng/λ		2.7		17.3		.040	167 ng/λ
9	Christopher Lipscomb								
10									
11	3 →	250λ							
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Gel Rans

PCR 31b (to confirm PCR 31 to test primers 395, 396) 6/14/8

	1	2	3	4 776	5	5847	5961	5968	9
1				X		2	3	4	
2	template			6		5	10	3	
3									
4	primers	395 ( )			221	2.17	2.17	2.17	
5		396 ( )			222	2.0	2.0	2.0	
6									
7	5x Buff			20		20	20	20	
8	DMSO			10		10	10	10	
9	dNTP's			6		6	6	6	
10									
11	H_2O					54.83	49.83	56.83	
12									
13						2.0 min extension 47°C			
14	2 Taq								
15	30 rounds								
16	47°C								
17									
18	Dilution of 221								
19		222-1							
20		395							
21		396							
22		303 (524)				Photo 6/15/8			
23		252 (51.2)				254			
24									
25									
26									
27									
28									
29									
30									
31									

Joel Ranier

PCR 33 (to test 395, 396) 6/15/8

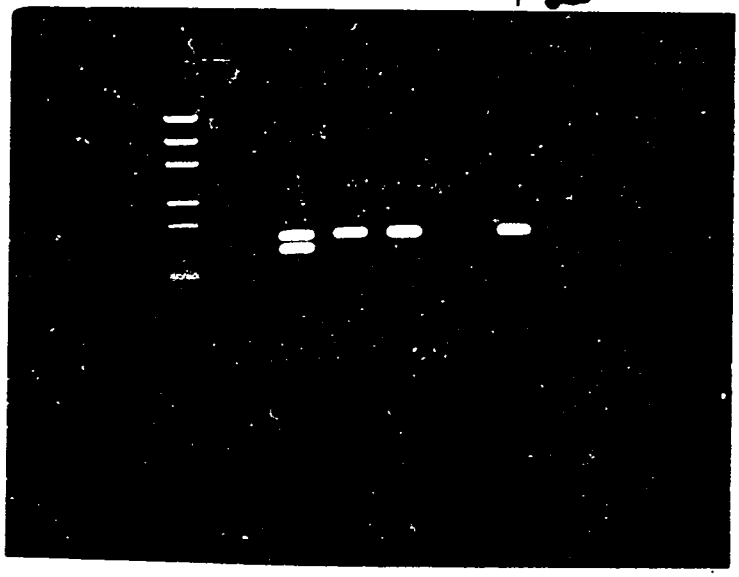
	1	2	3	4	5 776	6 776	7 776	8
1					1	2	X	
2	template				6	6	6	—
3	primers	221	(46)		2.17	—	2.17	
4		222	(49)		2.0	—	2.0	
5		257	(59)		~~1.7~~	—	1.7	
6		252	(51.2)		~~1.95~~	—	1.95	
7		376	(51 mM)		~~1.96~~	—	1.96	
8		303	(52.4)		~~1.91~~	—	1.91	
9		395	(56.3)		—	1.78	1.78	
10		396	(91.2)		—	1.1	1.1	
11								
12								
13	~~5~~ 2x Buff				20	20		
14	DMSO				10	10		
15	dNTP's				6	6		
16	H_2O				46.3	55.1	49.4	
17								
18						47°C		
19						2 min extension		

EFFICIENCY LINE: 22-206 AMPAD

Confirmation of 31

2 3 4 1 2

* 1 2



pos. con.

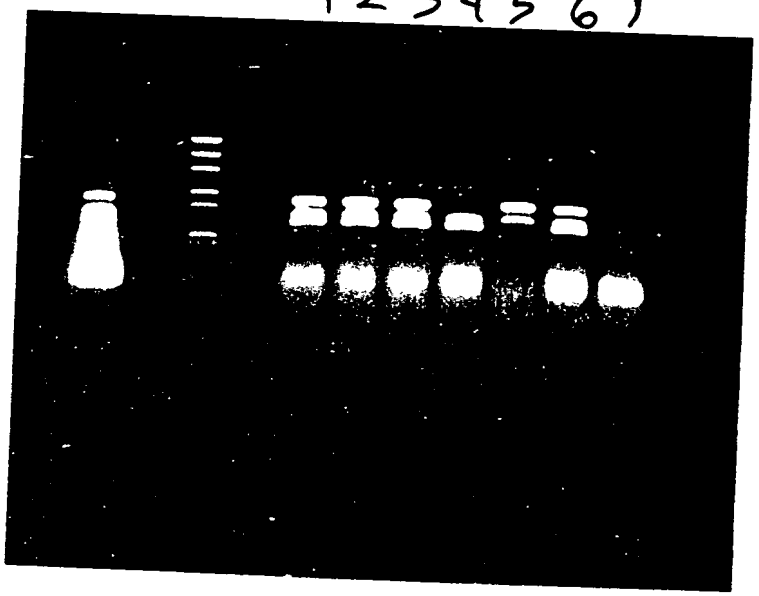
* R.G. cut purified digos out of gel & switched

31

Joel Ravi

PCR 34 (1) PND's and others 6/21/8

	1	2	3288	3989	2995	3987	748	3986	9
1			1	2	3	4	5	6	7
2	Hemylate		7.1	3.5	10.9	2.22	8.0	3.0	
3	H_2O		3.8	7.4		8.7	2.9	7.9	10.9
4									
5	Mix	1x	7x						
6	221	0.74	5.15						
7	222	0.66	4.6						
8	251	0.45	3.14						
9	252	0.48	3.37						
10	276	0.61	4.3						
11	303	3.6	25.1						
12	5x Buff	20	140						
13	DMSO	10	70						
14	dNTP's	6	42						
15	H_2O	46.56	325.92						
16		Add	89.1						
17									
18	2λ Taq Pol.								
19	3 min ext.								
20	47°C								
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



AMPAD · EFFICIENCY LINE · 22-206

	1	2	3	4	5	6	7	8 A_{260}	conc.
1	3988 ERIC DAVIS			698ng/λ		3.6	16.4	.158	
2								.017	70.8ng/λ
3	3989								
4	ROBERT SURET, JR.			1700ng/λ		1.80	18.8	039	142ng/λ
5									
6	2995								
7	DANIEL WEBSTER			634ng/λ		3.9	16.1	.110	
8								.011	46ng/λ
9	3987								
10	JOSEPH TORRANO			598ng/λ		4.2	15.8	.009	~~16.7ng/λ~~
11								.054	225ng/λ
12	748 (DRL)								
13	BRIAN BLY			645ng/λ		3.9	16.1	.336	
14								.015	62.5ng/λ
15	3986								
16	MORRIS AMNIO			307ng/λ		5.0	5.0	.178	
17								.090	166.7ng/λ
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Joel Ravier

PCR Automation Trials

6/22/8

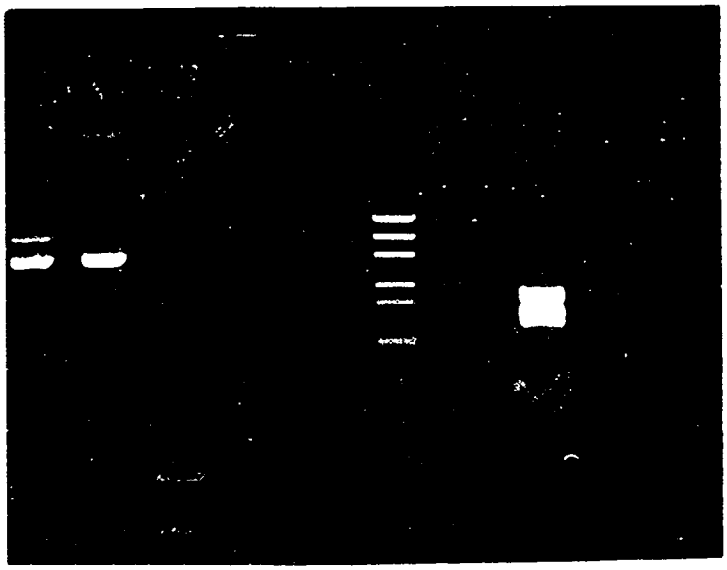
6/20/8

① No initial 7 min. denaturation
35 cycles
1.5 min denat.
1.0 min anneal 47°C
3.15 min extension 65°C
7 min extension at end.
(oil overlay) 50λ

(Picture see PCR 34 6/21/8)

② 7 min denat.
35 cycles
1.5 min denat. 94°C
45 sec anneal 47°C
3.15 ~~sec~~ min extension 65°C
7 min extension at end.
(oil 50λ)

PCR 2



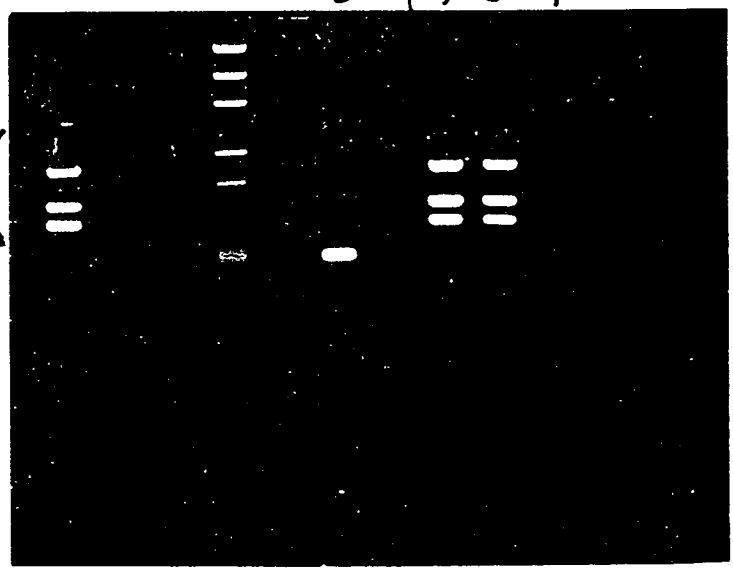
EFFICIENCY LINE™ 22-206

Joel Ravier

PCR 35 (to test new oligos 395, 396 + diagnostic cases) 6/23/98

	1	2 (ON 6/24)	X 1	X 2	5 3	6 4	7 5	8 6	9 7
1			776	776	776	776	#6	#4012	—
2	Template		6	6	6	6	1.8	3.16	—
3									
4	primers	221	0.14	2.17	|	|	2.17	2.17	2.17
5		222	0.06	2.0	|	|	2.0	2.0	2.0
6		251	0.45	1.7	|	|	1.7	1.7	1.7
7		252	1.95	1.95	|	|	1.95	1.96	1.95
8		276	1.00	1.96	|	|	1.96	1.96	1.96
9	(27.9)	303	3.96	3.6	|	|	3.6	3.6	3.6
10	(123)	395 ✓	3.80	.81	0.81		—	—	—
11	(91.2)	396	5.8	1.1	1.1	|	—	—	—
12	(91.2)	416	—	1.1	|	1.1	—	—	—
13	(618)	417	—	1.62	|	1.62	—	—	—
14									
15	5x Taq		20						
16	DMSO		10						
17	dNTP's		6						
18									
19	H_2O		41.99	40.0	56.1	55.3	48.8	47.5	50.6

3 4 5 6 7



ON PCR Machine
1:30 denat.
45 sanneal @ 51°C
3:30 extend

EFFICIENCY LINE™ 22-206
AMPAD

	1	2	3	4	5	6 DNA	7 H_2O	8 A_{260}	9 conc
1	Anthony Barrer				DRL #6	4.35	15.65	.088	283 ng/λ
2	575 ng/λ								
3									
4	Alan Sparkman			4012		4.96	15.04	.048	158 ng/λ
5	504 ng/λ								
6									
7	416-1							0.313	91.2 μM
8									
9	417-1							0.212	61.8 μM
10									
11	303/295							0.406	123 μM
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

O.D.'s of Oligo's 395, 396

	1	2	3	4	5	6	7	8	9
1				A_{260}		Conc			
2									
3	395-1			.186		56.3 μM			
4	395-2			.227		68.8 μM			
5	396			.301		91.2 μM			
6	303			.092		27.9 μM			
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Joel Ramos

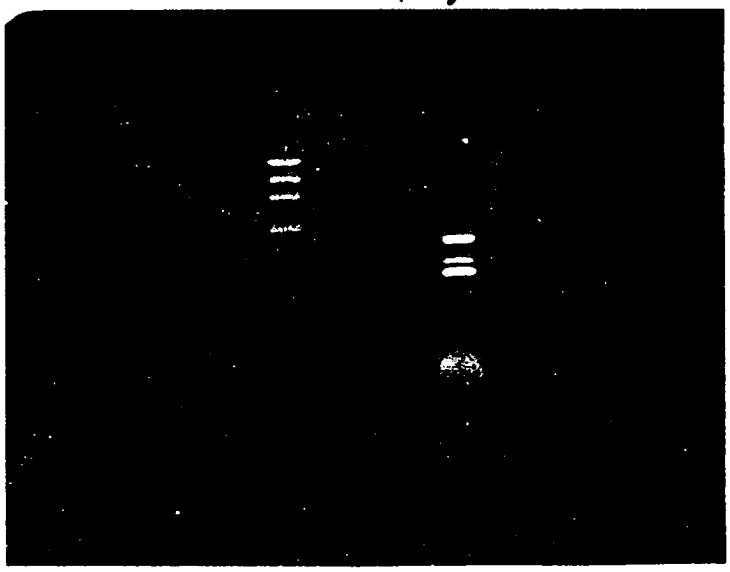
First Attempt [416, 417 (420, 421)] 6/27/18

			6λ
template			7.7λ
	dil	(27.9)	
primers	416	(110 µM)	3.6
	417	(8 µM)	
	dil	(17.2)	5.8
H2O			48.6

2' extension
37°C anneal
1λ Taq

(2) 3 sets + 395
396 on
Thermocycler

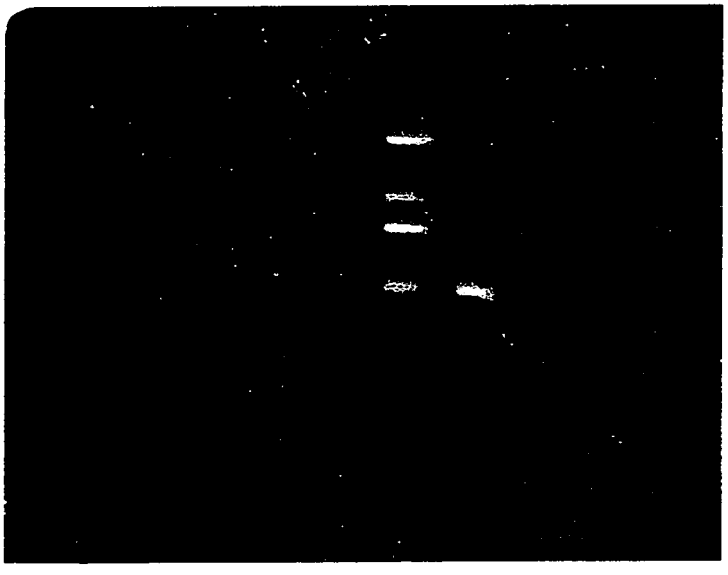
* Incorrect
primer conc.



Joel Ranier

PCR 36 (on thermocycler) 6/28/8

	1	2	3	4 1	5	6 2	7	8	9
1				776		776			
2	template			6		6			
3									
4	primers	221		0.75		—			
5		222		0.65		—			
6		251		0.5		—			
7		252		2.0		—			
8		276		1.0		—			
9		303		2.0		—			
10		395		0.80		0.8			
11		396		1.1		1.1			
12									
13	5x Buff			20		20			
14	DMSO			10		10			
15	dNTP's			6		6			
16									
17	H_2O			49.2		56.1			
18									
19									
20									
21	denat.	1:30							
22	anneal	45	51°C						
23	ext.	3:30							
24	2x Taq Pol								
25									
26									
27									
28									
29									
30									
31									



EFFICIENCY LINE 22-206 AMPAD

Dilutions of 416, 417, N. CTL - DNA 6/28/8

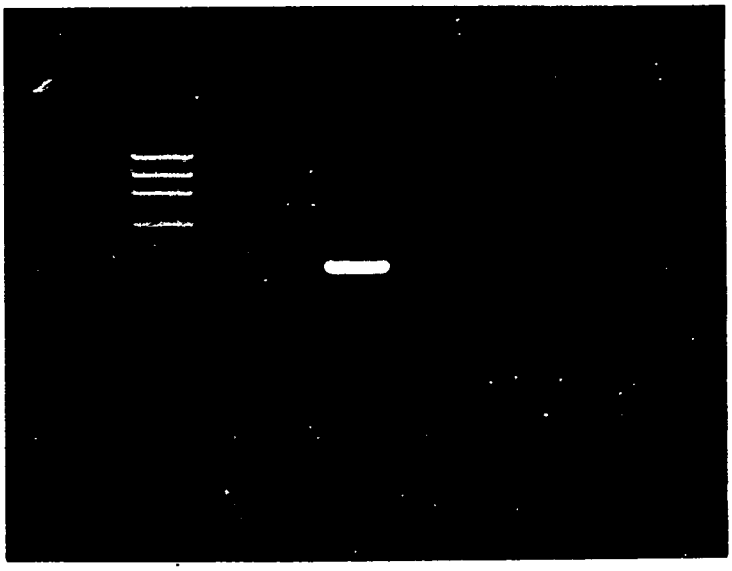
	1	2	3	4 H_2O	5	6 DNA	7	8 A_{260}	9 Conc.
1									
2	4162			~~39.3~~		~~10.7~~		0.401	116.8 μM
3									
4	417-2			~~25~~		~~25.1~~		0.171	49.8 μM
5									
6	416-2 dil		(116.8)	39.3		10.7		0.082	23.9 μM
7									
8	417-2 dil		(49.8)	25		25		0.092	26.8 μM
9									
10	416 2nd dil.		(416 stock 91.2)	36.3		13.7		0.09	26.2 μM
11									
12	417 2nd dil.		(416 stock 61.8)	30		20		0.077	22.4 μM
13									
14	N.CTL.		(1.16)	44.6		5.4		0.022	91.6 ng/λ
15									
16	257	(223)		46.4		5.6		0.099	30 ng/λ
17									
18	276	(163)		52.3		7.7		0.096	29 ng/λ
19									
20									
21	416	1:500							
22	417	1:200							
23									
24									
25									
26									
27									
28									
29									
30									
31									

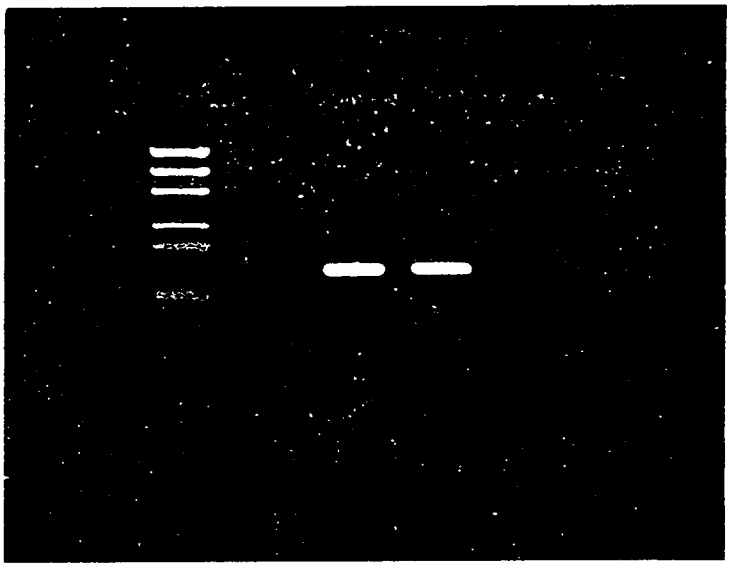
AMPAD · EFFICIENCY LINE™ 22-206

Pol. Rxn

PCR 37 [to test 420 421 (416, 417)] 6/29/8

	1	2	3	4 1	5 2	6	7	8	9
1				N. CTL.	N.CTL				
2	template (91.6)			5.46	5.46				
3									
4	primers	251	(30)	3.33	3.33				
5		252	(57.7)	1.95	1.95				(Heat 2)
6		416-2dil.	(23.9)	4.2	4.2		1		7/5 49C
7		417-2dil.	(26.8)	3.7					
8		416-2dil.	(23.9)	—					
9		416-2nd	(26.2)	—					
10									
11				20					
12				10					
13				6					
14									
15				45.36					
16									
17									
18									
19									
20	ON THERMO-								
21	CYCLER 49C								
22	51								
23									
24									
25									
26									
27									
28									
29									
30									
31									





AMPAD EFFICIENCY LINE® 22-206

Joel Rainish

PCR 38 (420, 421 against cloned DNA) 6/30/8

	1	2	3	4	5	6	7	8	9
1					λ DNA				
2	template				1 λ				
3									
4	primers	420	(23.9)		4.2				
5		421	(26.8)		3.7				
6		251	(30)		3.33			420, 421	run out
7		252	(51.2)		1.95				
8									
9					20				
10					10				
11					6				
12									
13					49.82				
14									
15									
16									
17									
18									
19									
20									
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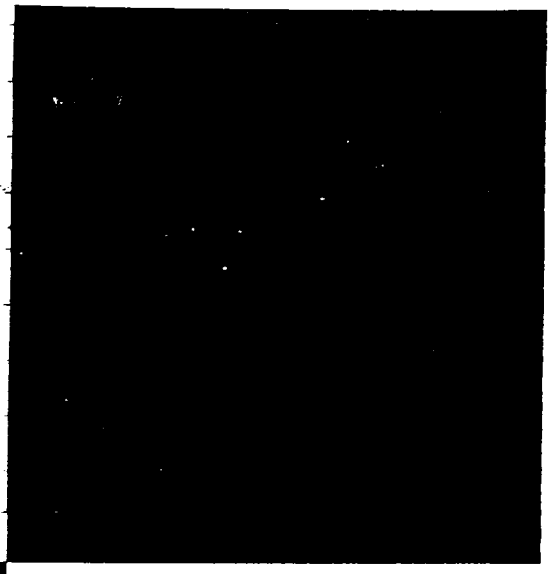
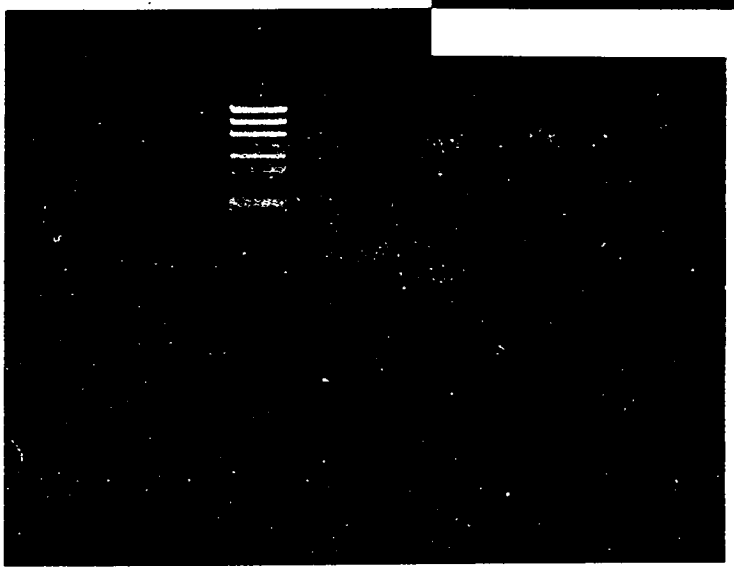
28									
29									
30									
31									





Joel Raine

Dilutions of Diagnostic DNA

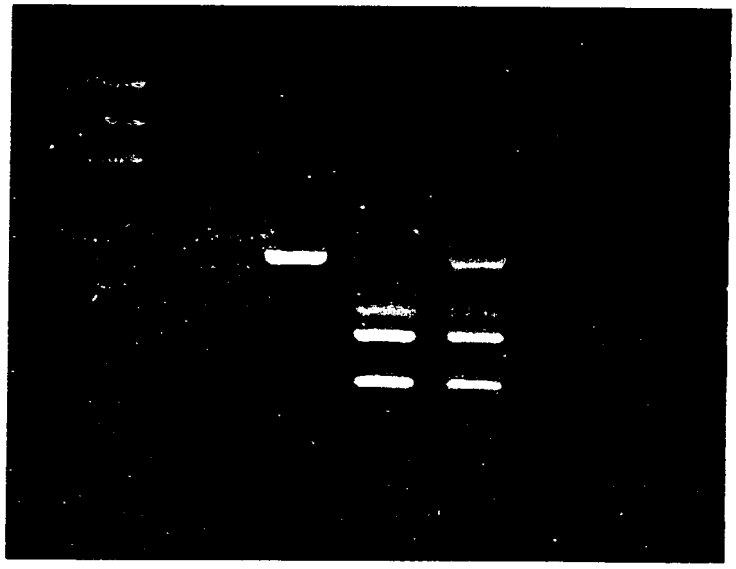
7/9/8

	1	2	3	4	5	6 DNA	7 H_2O	8 A_{260}	9 conc
1	4036								
2	Terry	Frazier		685ng/λ		3.6	16.4	0.031	129ng/λ
3									
4	4066								
5	John	Horner		661ng/λ		3.8	16.2	0.144	
6								0.020	833ng/λ
7	4023								
8	Ryan	Berube		598ng/λ		4.2	15.8	0.040	166.67ng/λ
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Joel Ravier

PCR 39 (Diagnostic) 7/7/8

	1	2	3	4	4086	4066	4023	8	9
1					1	2	3		
2	template				3.9	6.0	3.0		
3	H_2O				2.1	—	3.0		
4	primers	221	(1.36μM)						
5		222	(50.2μM)						
6		251	(30μM)						
7		252	(30μM)						
8		276	(29μM)						
9		303	(56.3μM)						
10		395	(123μM)						
11		396	(91.2μM)						
12									
13	Mix	1x	4x						
14									
15	221	.74	2.94 ✓						
16	222	.666	2.65						
17	251	3.33	13.32						
18	252	1.95	7.9						
19	276	3.45	13.8						
20	303	1.78	7.1 ✓						
21	395	0.81	3.25 ✓						
22	396	1.1	4.4 ✓						
23		20	80						
24		10	40						
25		6	24						
26	H_2O	44.18	176.72						
27									
28									
29									
30									
31									



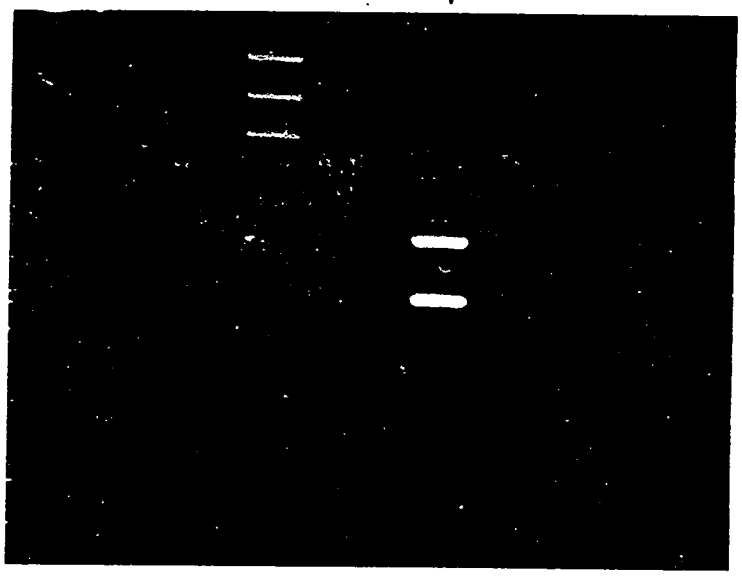
EFFICIENCY LINE 22-206

Joel Ranier

PCR 401 (to test remade 420, 421)

7/12/8

	1	2	3	4 N.CTL	5	6	7	8	9
1									
2	template			5.46					
3									
4	primers	251	(30)	3.33					
5		252	(51.2)	1.95					
6		465	(19.5)	5.1					
7		466	(17.9)	5.6					
8									
9	5 x Buff			20					
10	DMSO			10					
11	dNTP's			6					
12									
13	H_2O			42.56					
14									
15									
16									
17									
18									
19	41°C	machine							
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



AMPAD EFFICIENCY LINE 22-206

7/12/8

	1	2	3 A_{260}	4 conc	5	6 H_2O	DNA	8	A_{260}	conc
1										
2	465	28-mer	.303	123 μM		90.0	10.0		.048	19.5 μM
3										
4	466	26mer	.522	228 μM		44.5	5.5		.041	179 μM
5										
6	464	26-mer	.327	143 μM		41.3	8.7		.040	17.5 μM
7										
8	396	25-mer	.270	122.7 μM		39.8	10.2		.052	23.6 μM
9										
7/13/8	419	25-mer	.451	205		43.9	6.1		.053	23.6
11										
12	221	27-mer	.186	79.3		34.0	16.0		.059	24.8
13										
14	222	27-mer	0.200	840		35.1	14.9		0.055	23.1
15										
16	395	25		123		39.8	10.2		.059	26.8
17										
18	252	25		208		44.0	6.0		.051	23.2
19										
20	252	25		208		80	20.0		.109	47.2
21										
22	251	26		223		82.1	17.9		.109	47.6
23										
24	276	25		163		75.5	24.5		.092	41.8
25										
26	NCTL.			1.16		90	10		.024	150 ng
27	469								0.098	44.5
28	221	27mer							0.089	37.5 μM
29	396	25-mer							0.089	40.5 μM
30	465	28-mer							0.089	39.4 μM
31	466	26-mer							0.081	35.4 μM

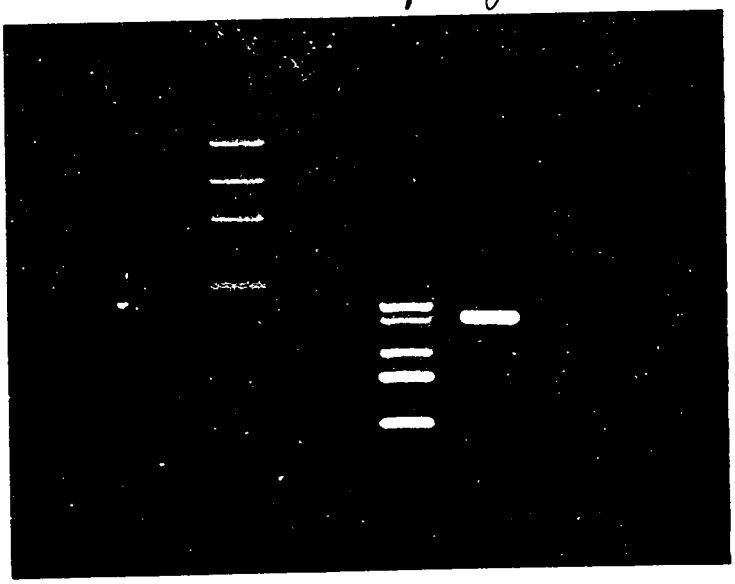
EFFICIENCY LINE = 22.206

Joel Ravier

PCR 41 (41 primers at 51°C)

9/13/5

	1	2	3	4	N. CTR	6	N CTR	8	9
1									
2	template				5.46				
3									
4	primers	221	136		.74				
5		222	150.7		.166				
6		251	30		3.33				
7		252	51.2		1.95				
8		276	23.6		~~4.35~~				
9		303	91.2		1.1				
10		395	123		~~3.45~~	0.81			
11		396	27.9		3.6				
12		465	19.5		5.1		5.1		
13		466	17.9		5.6		5.6		
14									
15					20		20		
16					10		10		
17					6		6		
18									
19					28.76		47.84		
20									
21									
22	51°C anneal								
23	3:30 ext.								
24	30 rounds								
25									
26									
27									
28									
29									
30									
31									



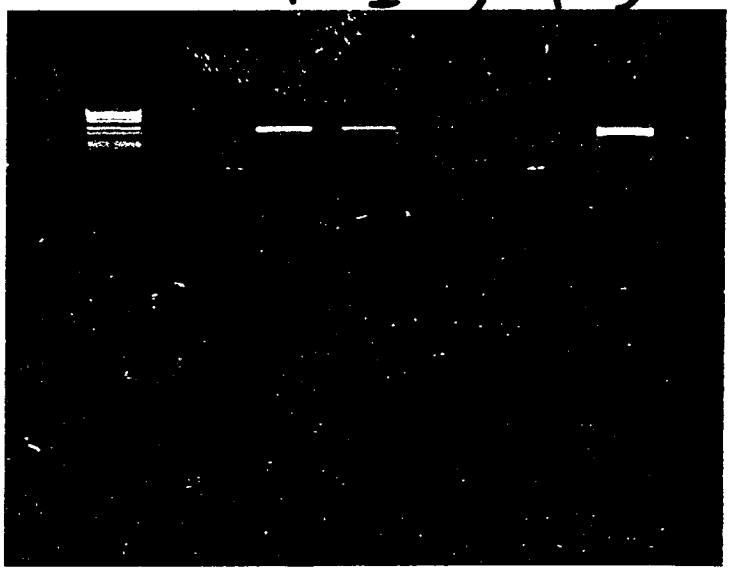
EFFICIENCY LINE 1-22-206 AMPAD

Joel Ranier

9/14/18

PCR 47 (to test 419, 469; mix/match 420, 421, 465, 466)

	1	2	3	4 1	5 2	6 3	7 4	8 5	9 6	X
1				N.CTL	N.CTL	N.CTL	N.CTL	Fetus	—	N.CTL
2	template			5.46	5.46	5.46	5.46			5.46
3										
4	primers	221	(24.8)					4.0		
5		222	(23.1)					4.3		
6		251	(30)					3.33		3.33
7		252	(23.2)					4.3		4.3
8		296	(29)					3.45		
9		303	(27.9)					3.6		
10		395	(26.8)					3.9		
11		396	(23.6)					4.25		
12	5'	420	(23.9)		4.2	—	4.2	—		
13	3'	421	(26.8)		—	3.7	3.7	—		
14	5'	465	(19.5)	5.1	5.1	—		5.1		
15	5'	466	(17.9)	5.6	—	5.6		5.6		
16		419	(23.6)					4.25		4.25
17		469	(17.4)					5.7		5.7
18	5x TaqBuff							20		20
19	DMSO							10		10
20	dNTP's							6		6
21	H_2O							41.0		41.0
22										
23	51° anneal 45s									
24	3:30 ext.									
25	30 rounds									
26										
27										
28										
29										
30										
31										



AMPAD EFFICIENCY LINE 22-206

Good Rower

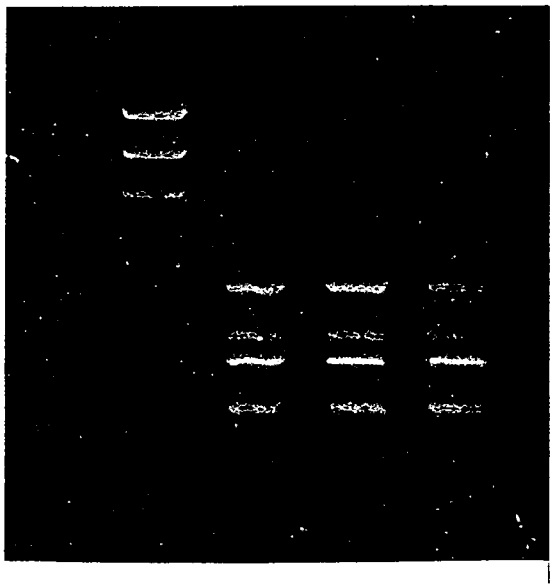
PCR 43 (All six + diagnostic) 9/15/18

	1	2	3	4 1	5	6 2	7 3	8 4	9 5
1				7.76		4062	3985	W. Am.	—
2	Template			6		4.7	3.08	3.2	—
3	H_2O			—		1.3	2.92	2.8	6
4	primers	221	24.8 μM	4.0					
5		222	23.1	4.3					
6		251	30	3.33					
7		252	23.2	4.3					
8		296	29	3.45					
9		303	27.9	3.6					
10		395	26.8	3.7					
11		396	23.6	4.25		51°C anneal 45s			
12		465	19.5	5.1		ext. 3:45			
13		466	17.9	5.6		2x taq			
14		469	17.4	5.7					
15		419	23.6	4.25					
16		H_2O		6.42					
17		Mix 1x	4x						
18		221 4.0	16						

(28) → (26) → 1 2 3 4 5

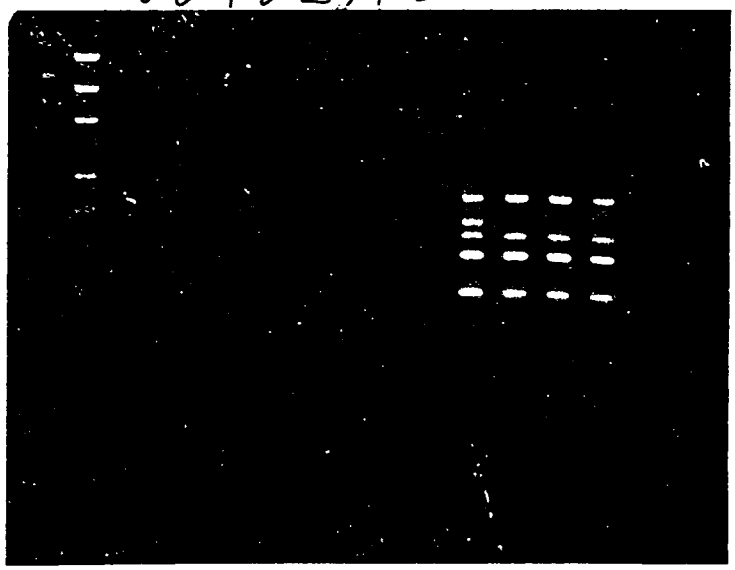
2 3 4 5 2 3 4 5

(223)



(123)

(228)



7/15/8

	1	2	3	4	5	6	7 DNA	8 H_2O	9 A_{260}	Conc
1										
2	4062	T.	Eddy	Amnio		259ng/λ	9.8	10.2	.091	
3									.079	492ng/
4	3985	A.	Roybal	Amnio		288ng/λ	8.7	11.3	.099	
5									.062	406ng/
6	(4)	Danna	Wallage	Amnio		180ng/λ	13.9	6.1	0.169	
7									.092	575ng
8										
9	4062								.026	162.5
10									.017	106.3
11	3985								.047	293.8
12									.026	162.5
13	W. Am.								.042	262.5
14									.025	156.3
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										

PCR-44 (to adjust conditions for 5th) 9/19/9

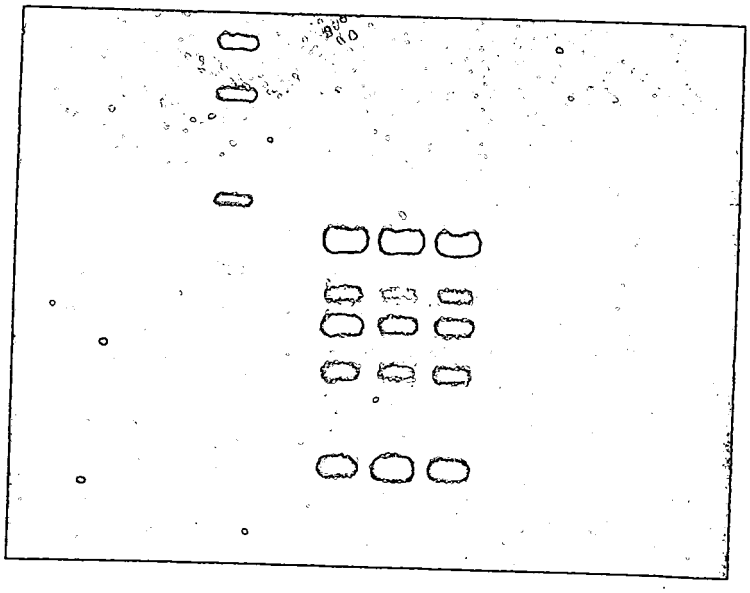
	1	2	3	4	5	6 2	7	8	9
1					N.CTL.	N.CTL.			
2	template				3.33	3.33			
3									
4	primers	221	(4.8)		4.0	4.0			
5		222	(25.1)		4.33	4.33			
6		251	(47.6)		2.1	2.1			
7		252	(47.2)		2.12	2.12			
8		276	(41.8)		2.4	2.4			
9		303	(56.3)		1.78	1.78			
10		395	(26.8)		3.7	3.7			
11		396	(23.6)		4.2	4.2			
12		465	(28.0)		3.6	3.6			
13		466	(27.1)		3.7	3.7			
14		469	(17.4)		5.75	5.75			
15		479	(23.6)		4.2	4.2			
16									
17	5x Buff				20	20			
18	DMSO				10	10			
19	dNTP's				6	6			
20									
21	H_2O								
22									
23	Ext. 4.0 min.								
24	(2) 3x Taq								
25									
26									
27									
28									
29									
30									
31									

EFFICIENCY LINE® 22-206

Joel Ranier

PCR 44 (diagnostic cases) (Evans) (Varma) (Clay) 7/25/8

	1	2	3	4	5	6 1	7 2	8 3	4
1						(225)	(112.5)	(231.25)	—
2	template					2.22	4.44	2.16	—
3	H_2O			1x	4x	2.28	—	2.34	4.5
4	primers	221	37.5	2.6	10.67				
5		222	~~23.8~~	~~4.3~~ 2.19	~~17.3~~ 4.76				
6		251	47.6	2.1	8.9				
7		252	47.2	2.1	8.4				
8		276	40.8	2.4	9.57				
9		303	26.8	1.7	7.1				
10		395	26.8	3.73	14.9				
11		396	40.5	2.5	9.9				
12		465	39.4	2.5	10.15				
13		466	35.9	2.8	11.3				
14		464	49.5	2.25	9.0				
15		419	23.6	4.2	16.95				
16	5XBuff			20	80				
17	DMSO			10	40				
18	dNTP's			6	24				
19	H_2O			26.09	104.36	+12.54			
20									
21									
22	3.5 ext.								
23	47°C								
24	Manuel								
25									
26									
27									
28									
29									
30									
31									



7/25/8

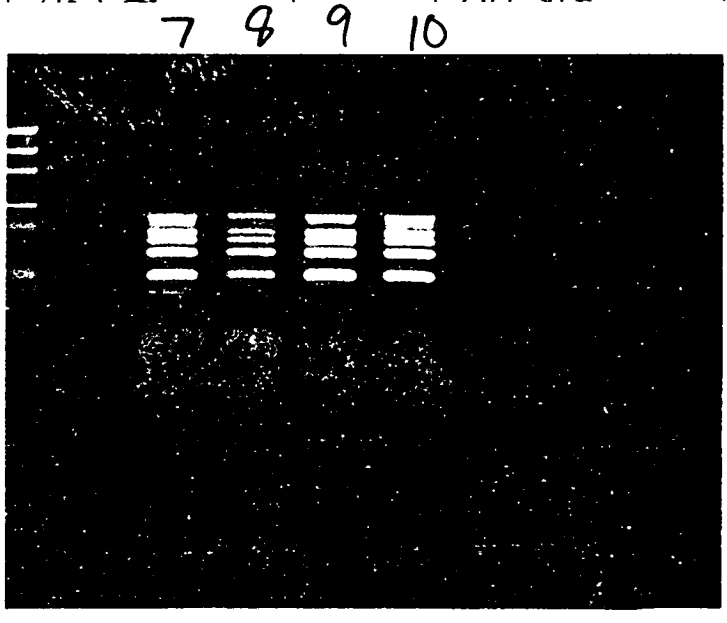
	1	2	3	4	5 DNA	6 H_2O	A_{260}	8	Conc.
1									
2	(1)	B. Evans (Am)		(524ng/λ)	4.8	15.2	.062		387.5
3							.054	(3-220)	225ng/λ
4	(2)	Asim Varma (affected)		(406ng/λ)	6.0	14.0	.018		112.5
5									
6	(3)	S. Clay (Am)		(274ng/λ)	9.0	11.0	.037		231.25
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

AMPAD EFFICIENCY LINE 22-206

Joel Ramos

PCR 44b (storage exper., diag. confirmation + control) 7/21/8

	1	2	3 8	4 9	5 10	6 1+2	7 3+4	8 5+6	9 7
1			B. Evans	A. Harmon	S. clay	(all 5)	-buff	-dNTPs	all CTL
2	template		2.22	0.95	2.16	N.CTL.	N.CTL.	N.CTL.	N.CTL
3			(225)	(112.5)	(231.25)	3.33	3.33	3.33	3.33 (did not add DNA ex.)
4	H_2O		2.28		2.34	1.17	1.17	1.17	1.17
5				1x	7x			10x	
6	primers	221	37.5	2.67	18.67			26.67	
7		222	84	1.19	8.3			11.9	
8		251	47.6	2.1	14.7			21.0	
9		252	47.2	2.1	14.8			21.19	
10		276	41.8	2.4	16.75			23.92	
11		303	56.3	1.78	12.43			17.76	
12		395	123	.81	5.7			8.13	
13		396	90.5	2.47	17.28			24.7	
14		465	394	2.54	17.77			25.38	
15		466	35.4	2.92	19.77			28.25	
16		464	44.5	2.25	15.73			22.47	
17		419	205	.49	3.41			4878	
18	DMSO			10	70			100	
19	DNA			3.33	23.31				
20	H_2O				2.69			2nd ctl	
21	Add	70.67	74.0						
22	To 1,2,+7 add		6x dNTPs, 2						
23	To 3+4 add		6x dNTPs						
24	To 5+6		20x buffs						
25	STORED	1	2 3 4 5 6	-70					
26									
27									
28									
29									
30									
31									

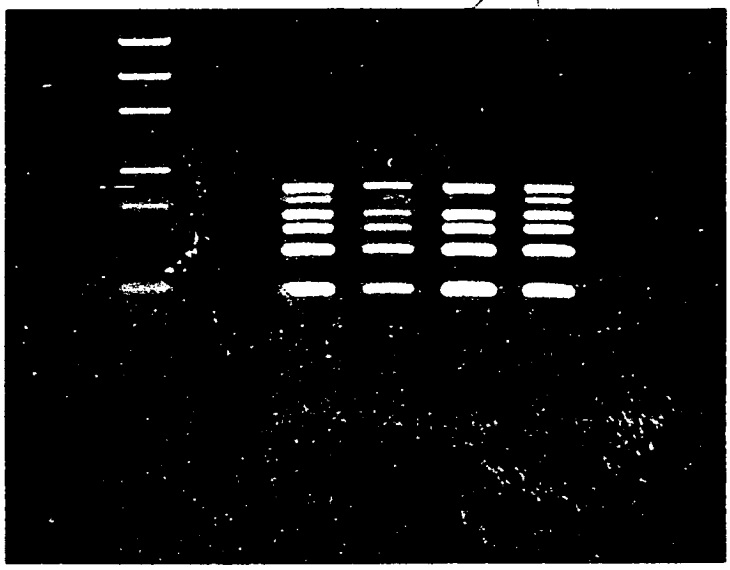


AMPAD EFFICIENCY LINE-22-206

Josh Kanter
JM Chan
7/28/93

PCR 44b

1 positive control
2 B. Evans (Amnio)
3 Asim Varma (Affected ♂)
4 S. Clay (Amnio)



547bp	47.4b	0.5	Hind
509bp	44.1	1.2, 3.8	"
462bp	30.2	3	Hind
415bp	30.2	1.7	Hind
360bp	9.7	7.5	Hind
268bp	47.4b	4.1	Hind

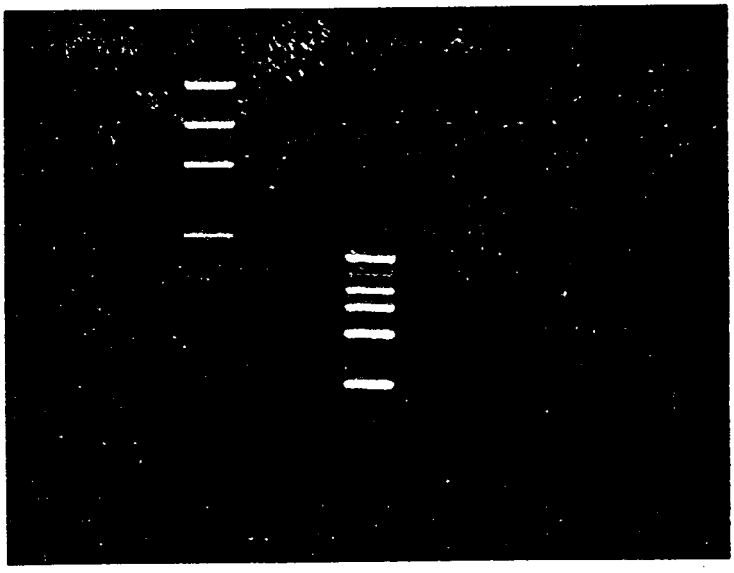
5/9C

Joel Ravier

PCR #45

7/28/98

	1	2	3	4	5	6	7	8	9
1					N. CTL				
2	template				3.33				
3									
4									
5		221	37.5		2.67				
6		222	39.1		2.55				
7		251	47.6		2.1				
8		252	47.2		2.1				
9		276	41.8		2.4				
10		303	56.3		1.78				
11		395	45.5		2.2				
12		396	40.5		2.47				
13		464	39.9		2.54				
14		466	35.4		2.82				
15		469	44.5		2.25				
16		419	40.6		2.46				
17	DMSO				10				
18	buffer				20				
19	dNTP's				1n				
20									
21									
22	Raised temp. to								
23	55°C anneal								
24									
25									
26									
27									
28									
29									
30									
31									



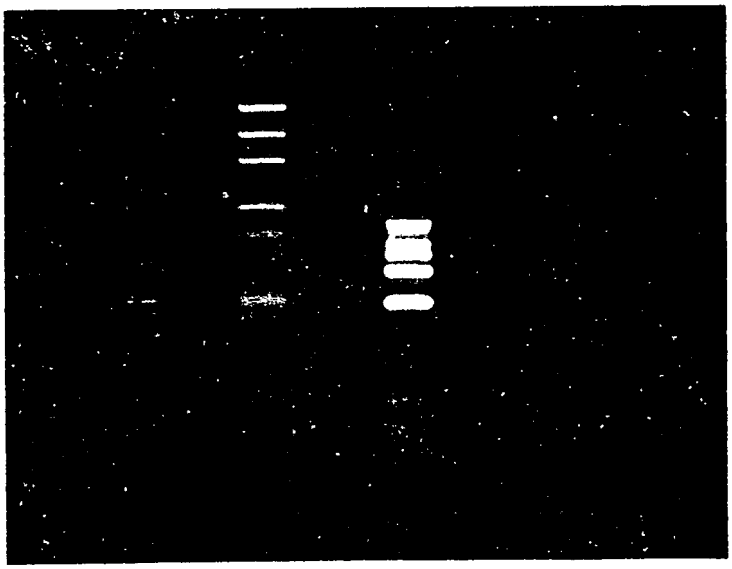
AMPAD · EFFICIENCY LINE® 22-206

Joel Ravier

PCR 46

7/29/18

	1	2	3	4	5	6	7	8	9
1							N. CTL		
2	template						3.33		
3				1x	3x				
4	primers	221	37.5	2.67	8.00				
5		222	39.1	2.56	7.67				
6		251	47.6	2.1	6.30				
7		252	47.2	2.12	6.36				
8		276	41.8	2.39	7.18				
9		303	56.3	1.78	5.33				
10		395	45.5	2.2	6.60				
11		396	90.5	2.47	7.40				
12		465	39.9	2.54	7.60				
13		466	35.4	2.82	8.47				
14		464	44.5	2.25	6.74				
15		419	40.6	2.46	7.39				
16									
17	Buff			20	60				
18	DMSO			10	30				
19	dNTP's			6	18				
20									
21	H_2O								
22	Add	96.67							
23									
24				35					
25				rounds					
26									
27									
28									
29									
30									
31									

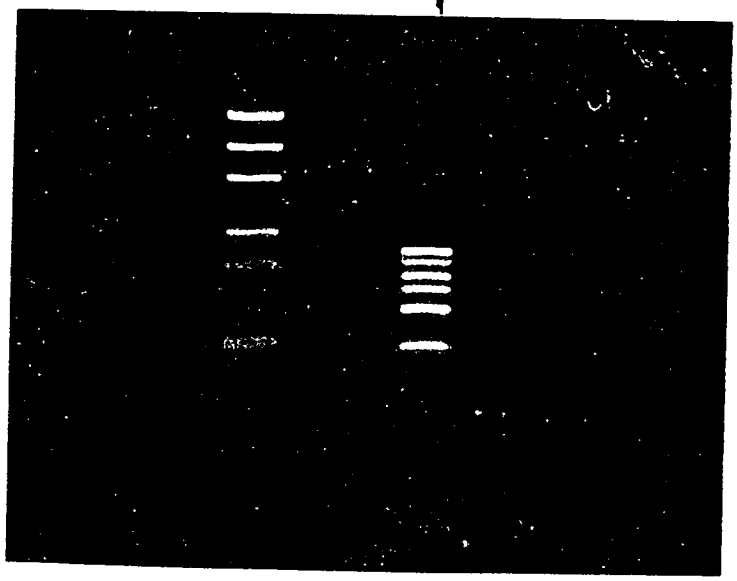


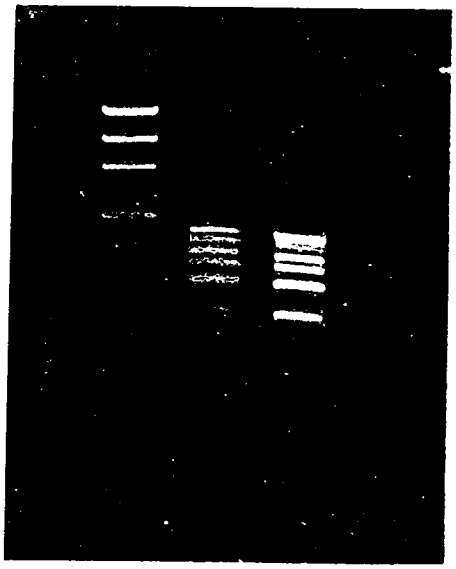
EFFICIENCY LINE™ 22-206 AMPAD

Joel Ramler

PCR 47 (to raise conc. of 44.1 amplicon) 7/31/8

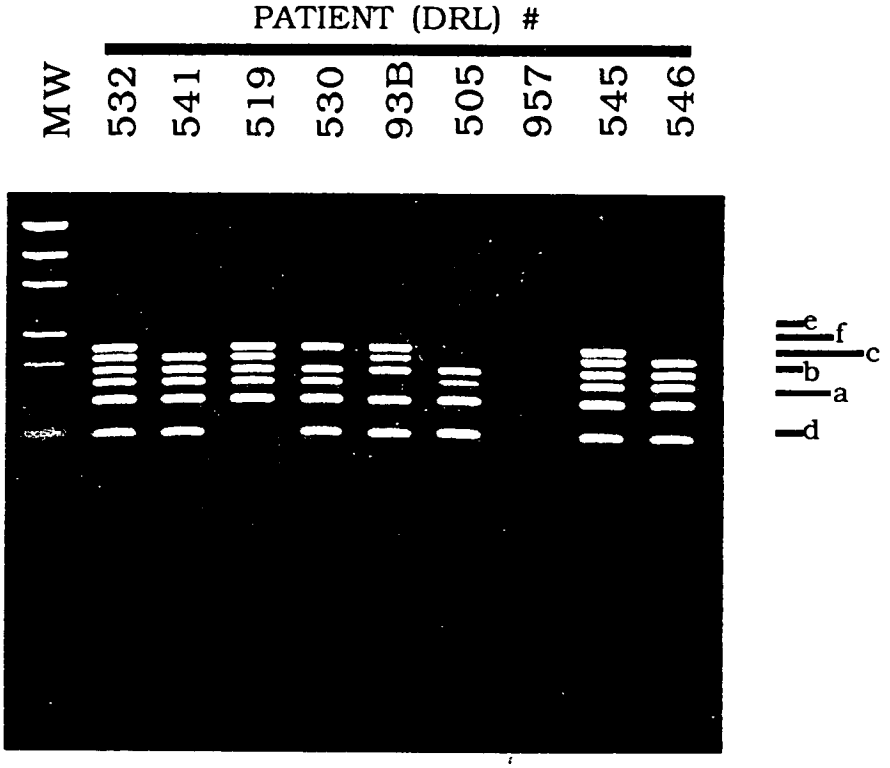
	1	2	3	4	5 1	6 2	7	8	9
1					N.CTL	N.CTL			
2	template				3.33	3.33			
3									
4	primers	221			2.67	"			
5		222			2.56	"			
6		251			2.1	"			
7		252			2.12	"			
8		276			2.39	"			
9		302			1.78	"			
10		395			2.2	"			
11		396	(44.5)		2.25	"			
12		465			2.54	3.81			
13		466			2.82	4.23			
14		464			2.25	"			
15		419			2.46	"			
16									
17	buffer				20	"	50% more 465,466		
18	DMSO				10	"			
19	dNTPs				6	"	(20λ) (10λ)		
							2^{35} 2^{30}		

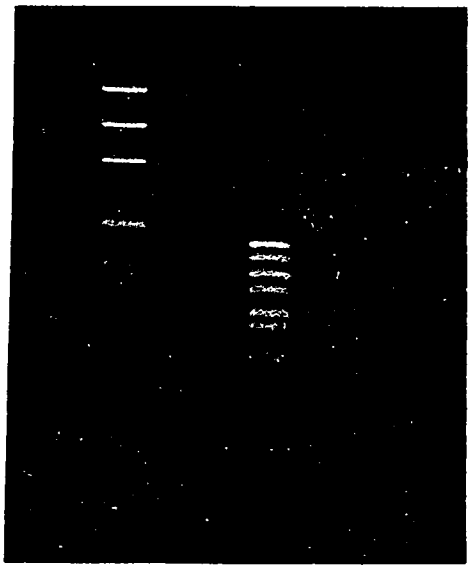




25 rounds
20λ
55°C

Joel Kanter





Re-screen of 44-1 gen. clones 5/17/8

Hong's screen of 4x4 Library λDASH

- need to re-purify 5 picks:
  1C, 2A, 7B, 9A, 9S
- plate 10 + 100λ of 2° picks
  (2nd pick of 20 - row 3 Hong's box)
  got 1-100 on 10λ plate
  lift all except for 1 too lo + 1 too hi
- pre-hyb O/N 50% F

Label
  44-1 ~20ng 1/2 rxn 11:15 →
  0.6 HcII frag 63-1 ~20ng " "

Hyb in 40% F

TCA↓ CPM:
1λ/100λ: 44-1 → 20x10⁴ cpm
0.6 → 28x10⁴ cpm

lifts - 7 filters
  12mls - used 60λ 44-1

also hyb Joel's blots of his 5 cDNA clones
  1) cut c̄ SstI-Kpn 2) cut c̄ EcoRI
⊕ with 63-1 2.5kb Hc - check for hyb to 0.6kb Hc II 63-1
  10mls 40% F c̄ 50λ probe

PCR CASES DMD 5/18/8

<u>family</u>

- Russel Jordan

483 Jordan - Aff. male XJ1.1 + 9.7 deletion
male fetus not affected
- do aff. male + fetus only

43B Ruggiero Aff male (Nicholas Posay) - 449, 638
87.1-15 deletion
male fetus unaffected - 3653, 3666
- do fetus + aff. male

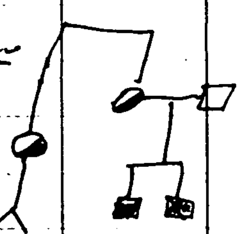
<u>ODD severe becker deletions</u>

- Randy + James Branche 3565 + 3566

469 Roussem 2 Bros. c̄ severe BMD/mild DMD
deletion of 9.7 Exons 3-7
but funny pattern at 7.5 kb band
similar to 2nd family
PCR for involved exon only + 0.5HB [illegible]

→ aff. male c̄ same deletion DNA #1504

Roussem



3647

also 3rd case c̄ similar 9.7 deletion + same funny pattern
AT 7.5-8.5 kb : Watkins, Sharon ♀ (3685); Alfred (aff ♂) (3758)
+ fetus (3722)

<u>Previous PCRS</u>

1st PND - Tayin UCLA manuscr: 461, green [Craig Spinneto aff. male]
♂ fetus not deleted

2nd twins ♀♀ 396 Arecio - normal pattern

1µg

MEMO

Jeff X4777 needs 0.5µg DNA on below. – (Please call him when ready)

438 Nicholas Posey 449, 638

✓ Male fetus Ruggiero – recd 1988.
3653, 3666

469 Bruneau, Randy OR James. 1µg/2 3565, 3566

Lynn Rousseau – 3560, 3561

✓ Male fetus Rousseau 3647

✓ Mary Richardson 3549, 3552

485 ✓ Watkins, Sharon 3685

✓ Watkins, Alfred – 3758

✓ Watkins, Male fetus 3722?, 3796? Same

grendel CR

Deletion Summary multiplex figure 5/19/88

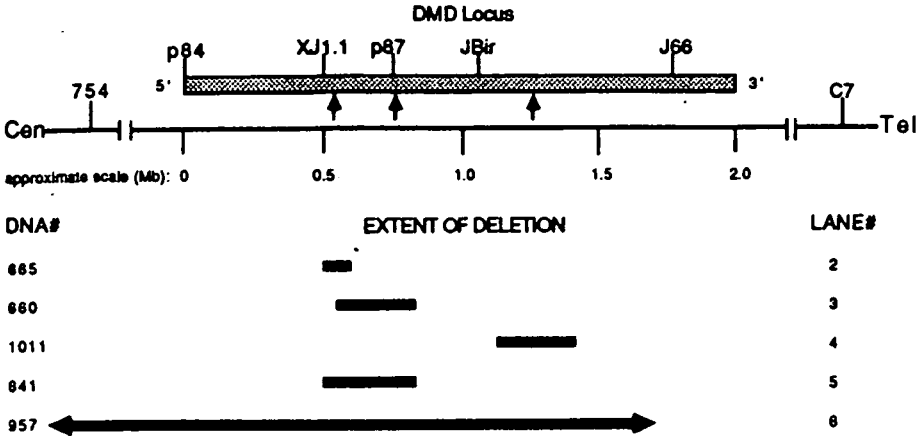
	1	2	3	4	5	6	7	8	9
1									
2	DRL#	DNA#	NAME		deletion				
3	42	665*			9-7-730-2	7.5, 10.5, 4.2 only ⊕XJ1.1			
4	93	660*	Hadicon		⊕XJ1.1; 7.5; Δ30-2: 10.5→87.15; ⊖87-27, 25, 30				
5	~~184~~ 156	1011	Eric Fronick		Δ47.46-44-1.2	0.5-1.6		BmD	
6	24	641*	Scott Jones		+XJ1.1; Δ9-7-30-2:	7.5→87.15; ⊕ 87-27, 25, 30			
7	?	957*	John Brandt		Δ754-J-Bir, Δ 0.5q 47.46 3' breakthrough				
8		776			pos. control for region of interest; ?→				
9									
10		* = Tested with XD-1							
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

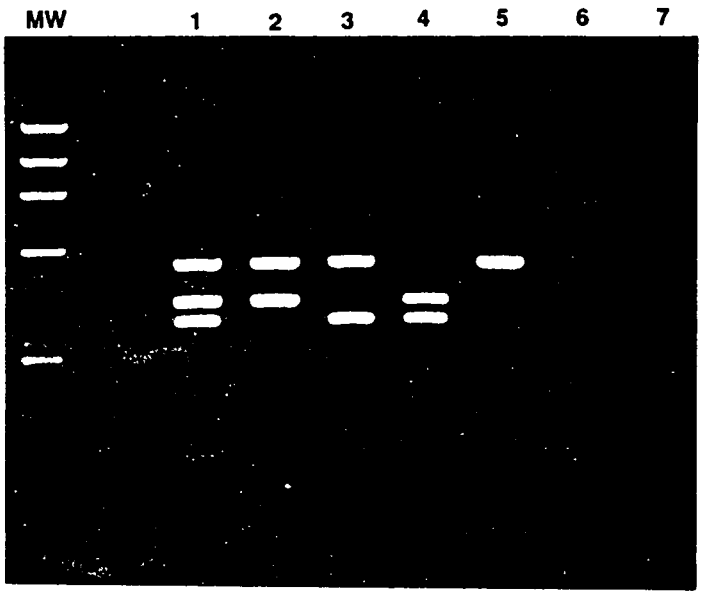
5/20/88

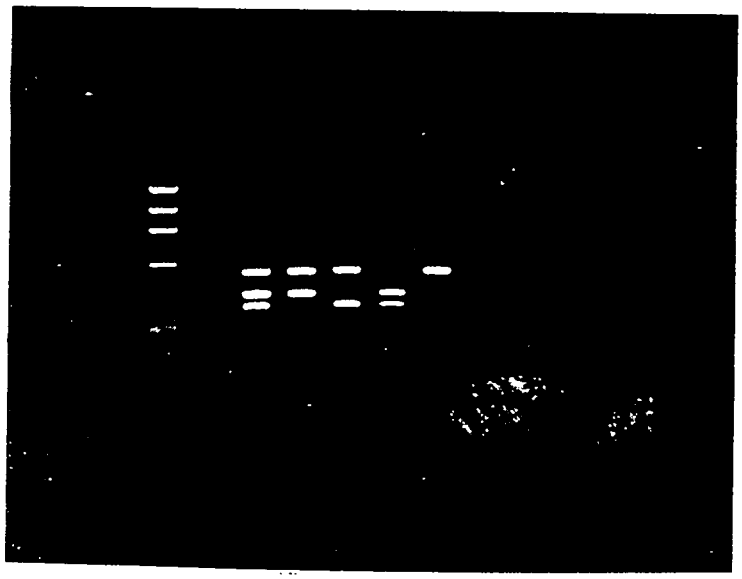
EFFICIENCY LINE® 22-206 AMPAD

	1	2	3	4	5	6	7	8	9
1		776				pos. control			
2	DR #42	665		- marked		+		(-7.5, 10.5, 4.2)	
3	DR #93	660		- 87.15	Haddon		-10 +7.5		
4		1011		- 0.5	Eric Crimi K, DR	#156		BAD	
5	DR #24	641		Scott Jones	- marked, - 87.15			(-10, 7.5)	
6									
7									
8		✓ JB	all 3	DNA #957		BRAZAEL		Δ 754, ΔJ-BiR	
9									
10									
11									
12	(156)	✓ 1011	deleted	474B 0.5 → 1.6					
13									
14		✓ (93)	7.5 ⊖ / 10.5	→ ? -87.15; +27 87.25 +30					
15			⊕						
16		✓ (42)		Δ 7.5, 10.5, 42 only					
17									
18		(24)	⊖4.6	/ ⊖7.5 → ⊖87.5; ⊕87 27, 25, 30 →					
19									
20									
21		754	XJ1.1	mm	1	PERT 8	15	27 30	J.BiR
22	1011								+ +
23	93			+				+	
24	42		+						
25	24		+					+	
26	JB → DNA #957								
27									
28									
29									
30									
31									

5/20/88

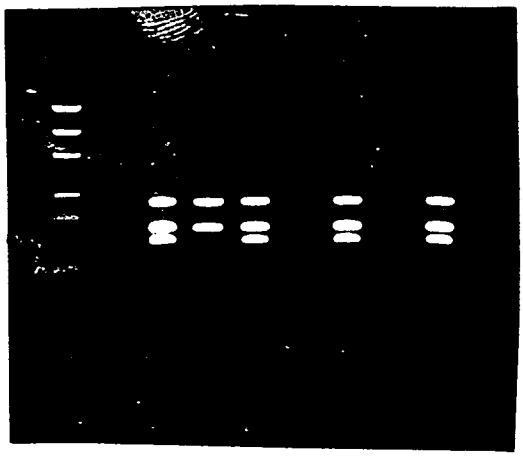

DMD Locus
p84
XJ1.1
p87
JBir
J66
754
5'
3'
C7
Cen
Tel
approximate scale (Mb): 0
0.5
1.0
1.5
2.0
DNA#
EXTENT OF DELETION
LANE#
665
660
1011
841
957
2
3
4
5
6


MW
1
2
3
4
5
6
7



5/24/88

MW	DRL# 483			X	DRL# 43B		
	CF	AM	MF		CF	AM	MF



6/3/85

Joelle –

→ PCR on all 4ff. male DNA —

473 Surrette
484 Miller
505 Young
513 ~~Leonard~~ Caputo
514 Reisbeck?
519 Van Zandt
520 Stone
521 Worth.
522 Cox
523 Creighton
524 Buchanan
228 Derry
531 Roybal

See me
D. Gaskey

PAT WATCH

6/2/88 DNA samples for PCR on DMD males

	DNA #	NAME	DRL #
* 1st Priority Group	346	George Derry, Jr.	22B
	472	Thomas Davis	70B
	3934	Shane Worth	521
	3955	Alan Cox	522
	3920	Douglas Hazelton	523
	3944	Billy Buchanan	524
	3948	Andrew Royse	531
	3929	Keith young III	505
	[redacted]	[redacted]	[redacted]
2nd priority Group	3940	Scott Miller	484
	3860	Donald Capello	513
	3880	Wayne Noon	514
	3895	David Van Zandt	519
	3950	Matthew Stone	520

6/7/88

Dot-blot hyb. of 44.1 3.8 kDMD cDNA clones

1. 5λ mini-prep DNA , pUC as control.
   - add 4λ 5M NaCl
   - add 1λ 1M NaOH
   - boil 2', ice
   - add 1λ 100% etH.

2. Pre-wet nylon membrane ē H_2O
   - spot DNA on filter , 5λ/station
   - ~~dip~~ wash gently in 2×SSC
   - air dry

3. Pre-hyb 1hr, 50% F, add extra SDS to 1%

4. Hyb ē 44-1 ~3 hrs.
5. wash, hi-string.
6. expose to film.

6/8/88

1 2 3 4 5 6 pT2

44-1 3.8kb N3 cDNA

vs. 44-1 cDNA

15hr. exp.

Labelling of 44-1 Oligomers

7/6/88

40 mers not purified

		1/5000 dil. 260	280	260/280	1/5000 conc. mg/ml	total yield
442	10kb exon	.496	.276	1.80	.016 mg/ml	4mg.
443	3.1kb exon	.444	.222	2.00	.015 mg/ml	3.66 mg
444	3.7kb exon	.496	.275	1.80	.016 mg/ml	4 mg

dissolve in 50λ TE (1/2 syn. off column) dilute 1/500, + 1/5000

1/5000 dilution each is 15-16 ng/μl (ng/λ)

26ng = 1pM end.
5pM end = 130ng = 8-9λ

Label each

DNA	3λ
10X kinase	1λ
T4 kinase	1λ
γ-ATP P^{32}	2λ
H_2O	4λ
	10λ

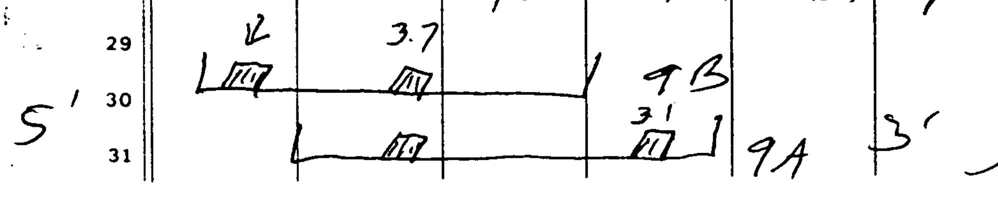
after 1hr 37° add 90λ formamide dye + load 4λ on a 12% acryl. gel - save rest for hybs.

Hyb to old blots of Hong clones 9A + 9B

443 - Lit up 9A Eco band that lit cDNA nothing on 9B (3.1kb H3)

444 - Lit up new Eco band on both 9A + 9B wasn't lit cDNA, different than 9B cDNA band (3.7kb)

442 - none lit up (10kb)



possible exon structure

exons cloned 7/12/88

5' → 3'

	B.P. Amp'd		
①	360	1) 9.7 =>	7.5 kb HindIII exons
②	415	2) 87.15 adjacent exon [30.2, 7.5 kb H3]	exon 17
	462	3) not Tested yet, 87.25 adj exon [30.2, 3 kb H3]	exon 19
④	1.268	4) 47.45 4.1 kb H3	
③	1.547	5) 47.45 0.5 kb H3	
	506	6) (partially tested) 44.1 [1.2 + 3.8 kb H3]	

cloned, sequenced, no oligo's yet

11) exon 12 (30.2, 4.2 kb H3)

cloned, being sequenced

A) 44.1, 1.6 kb H3
B) 44.1, 3.1 kb H3
C) 44.1 3.7 kb H3 - prob. not confirmed

freq. of detection

1,2,5,12,3 = 35% of all, 45% of last
1,2,4,5 => slightly higher
1,2,4,5,16 => 75%
1.4 => 50%

1/10,000 dilution | OM 471, 472, 473 | Seq primers 44-1 region

	260	280	260/280
471	.057	-.010	2.03
472	.114	.054	1.30
473	.099	.050	1.12
BL	0.000	-.038	

	0	-.042	260/280	conc.	yield
471	.141	.041	1.58	4.65 ug/ml	4.65 mg
472	.171	.072	1.43	5.64 ug/ml	5.6 mg
473	.120	.048	1.33	3.96 ug/ml	3.96 mg

dissolved in H_2O; 1/10,000 dilutions prepared for sequencing

9A + 9B prep 1/4/8

- plate lysates titered 7/13/8 by percy

9A ~ 4×10^7/ml

9B ~ 8×10^7/ml

replate ~ 100k/plate

2:1 9B, 4:1 9A

ACCAACCTCTAGCAATATCCATTACC

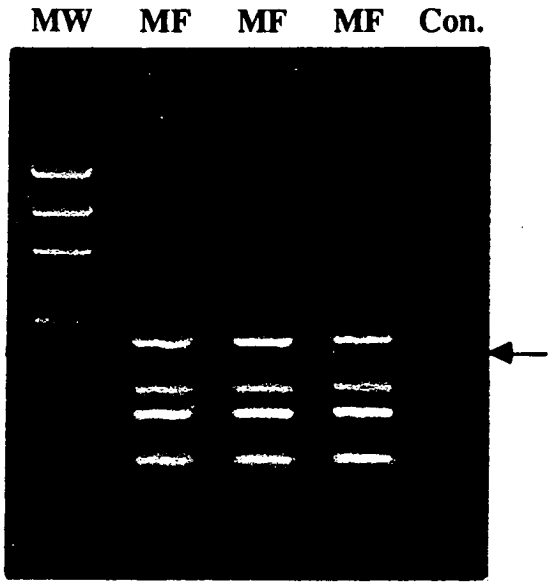
CAG
GTC

TCTTGGACCTXATA

PRENATAL DIAGNOSIS of DMD GENE DELETIONS

-Multiplex Amplification Using Primers Flanking Five DMD Exons

-Template DNA Prepared From Amniotic Fluid Cells

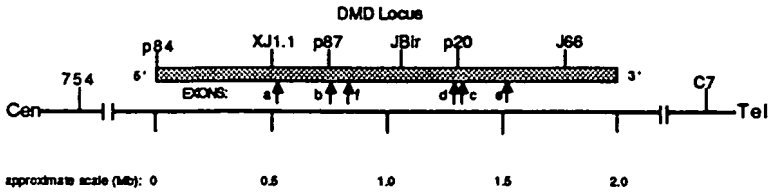


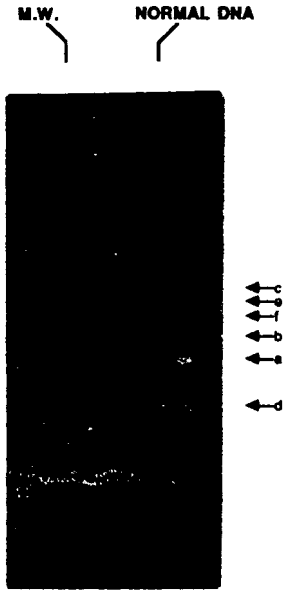
Diagnosis: DLN DLN NORM

1/18/8

MULTIPLEX DNA AMPLIFICATION AT THE DMD GENE

-Primer sets flanking six exons

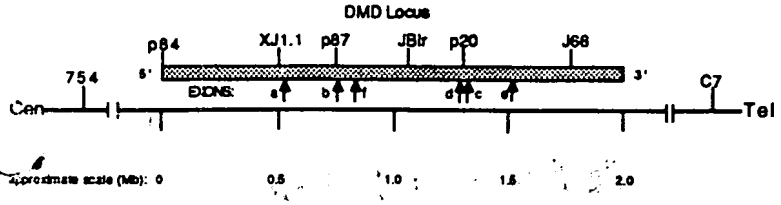


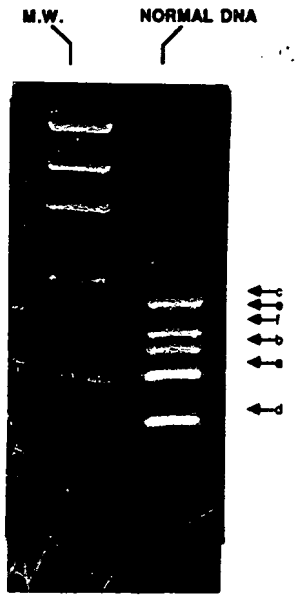


7/15/98

MULTIPLEX DNA AMPLIFICATION AT THE DMD GENE

-Primer sets flanking six exons





Labelling of pXD-1 for Hu. Gen. Lib.

[redacted]/87

F_1

$235896 \div 3 \times 1000 \times 2 = 1.6 \times 10^8$ cpm

F_2

$49253 \div 3 \times 950 \times 2 = 3.1 \times 10^7$ cpm

4.7×10^9 dpm/ug

T=002.00 A=235895.0(0.3%) B=235862.5(0.3%)

F_1

T=002.00 A=049250.0(0.7%) B=049244.0(0.7%)

F_2

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LABELLING OF XJ10 (to screen Hu. Gen. Lib.)

[redacted]/87

F_1

$54{,}402 \div 3 \times 1100 \times 2 = 4.0 \times 10^7$

F_2

$17{,}988 \div 3 \times 600 \times 2 = 7.2 \times 10^6$

2.36×10^9 dpm/μg

7/30

Screening of Hu. Gen Lib. w/ XJ10 in progress

Washed filters → 2x SSC, 0.1% PPi, 0.1% SDS
for 2x 5 min. RT
2x 20 min 60°C
0.5x SSC (diluted 2x SSC...)
for 15 min, 45°C

On Film

Also rescreened 2° from Mouse cDNA Lib. (w/ XJ10)
Titer of pK19.4 from Hu. Gen. Lib. vs pXJ41 = $2.0 \times 10^{4/m}$
Plated out ~2000 pfu (100λ of undiluted pk)

- Since transformation of DH5α w/DMD clones was unsuccessful competent T6-1's were prepared and transformed
- Amplified 4° and plated stock (12.1 & 12.2 from Hu. Gen. Lib. vs. XD-1) Add 5ml SM, shake gently ~2hrs

12.1
titer = 1.8×10^6/ml
$50 / 1.8 \times 10^6 = .028\mu l$
$1/10^3$ dilution, take $2.8\mu l$

12.2
titer = 4×10^5/ml
$50 / 4 \times 10^5 = .125\mu l$
$1/10^3$ dilution, take $1.25\mu l$

- Washed filters from plate 19 (high density) XD-1, last wash 0.5×SSC
- replated NT.1 (from HGL vs XJ10)
- photographed gel characterizing XJ10

Titering Amplified (pure) stock Phage [redacted] 87

from Hu. Gen. Lib. vs. pXD-1

12.2 10^{-3}, 28λ

1 - 21
2 - 37
3 - 36
4 - 49
total 143

$143 \times 35.7 = 5.1 \times 10^3/ml \times 10^3 = \boxed{5.1 \times 10^6/ml}$

12.1 10^{-3}, 125λ

1 - 34
2 - 56
3 - 37
4 - 78
total 205

$205 \times 8 = 1.64 \times 10^3/ml \times 10^3 = \boxed{1.64 \times 10^6/ml}$

12.2

$3 \times 10^5 \div 5.1 \times 10^6 \simeq 60\mu l$

12.1

$3 \times 10^5 \div 1.64 \times 10^6 \simeq 180\mu l$

- no positives on film w/ 19.1, 19.2, 19.4 vs. XD-1
- ~~cell~~ TG-1's transformed by DMD clones
- plated out 300,000 pfu onto lg. Amp plate
- eluted phage - 12.1, 12.2
- lifted from replating of NT.1
- made 200mls hyb. (40%F), no H.S. DNA
- made Neutralizing solution (1.5 NaCl, 0.5 Tris HCl pH 7.5

Labelling of XJ10; Isolation of DNA 12.1, 12.2 [redacted] 87

½ rxn.

XJ10

F_1

$30601 \div 3 \times 1100 \times 2 = 2.2 \times 10^7$ cpm

20,000 cpm/µl

F_2

[redacted]

Eluted 9.6mls from 1g plates w/ 12.1 and 12.2
Spun down debris and removed supernatant
Added 1ug/ml DNAse I and RNase A - 37° 30'
PEG precipitated, aspirated supernat. + to remove
Respun and removed remaining PEG w/ pipet
Resuspended in 1ml SM, tranferred to microtube
Spun to remove debris
Transfered to blue tube, diluted to 2ml w/ STE
Added 1/20th vol 10x SET (100)) Add prok to .1mg/ml
Extracted w/ phenol, chlor/phenol, chlor.
Added 2 vol. EtOH - let sit in freezer
over night

Filters NT.1,2,3,20 on film Completed [redacted] /87

Digestion / Isolation of 12.1, .2

Spun EtOH precipitated DNA, removed supernatant
Washed w/ 70% EtOH, removed supernatant
Pooled 1st EtOH supernatant w/ TE + Am Ac + 3mls EtO
Put at 37°C
Spun at 10K for 15 min.
Washed w/ 70% EtOH
Dry Vac for 20 min.

Am Ac was added along with the TE, not resuspended 1st
Heating for 5 min at 65°C to aid in resuspension
Most of DNA resuspended, put at 65°C for a couple more minutes
Added 3mls EtOH
37°C 10', 65°C 5'
Spun at room temp. 15'
Resuspended in 0.5ml TE
Spun briefly
Transfered to eppendorfs
Placed in Box I as phage lysates 12.1, 12.2

(12.1) 2.3 mg/ml

(12.2) 2 mg/ml

Analysis of 12.1 and 12.2 by Restriction Digest

	DNA	(µl)	10x Enzyme Buffer (µl)		Enzyme (µl)		TE (µl)
1	Marker	20					
2	12.1	4.3		B*	5	BamHI	5	30.7
3	12.1	4.3		H	5	Sal I	5	30.7
4	12.1	4.3		E*	5	EcoR I	5	30.7
5	12.2	5		B*	5	BamHI	5	30
6	12.2	5		H	5	Sal I	5	30
7	12.2	5		E*	5	EcoRI	5	30
8								
9	Spermidine		2X					
10	RNase A		3X					
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
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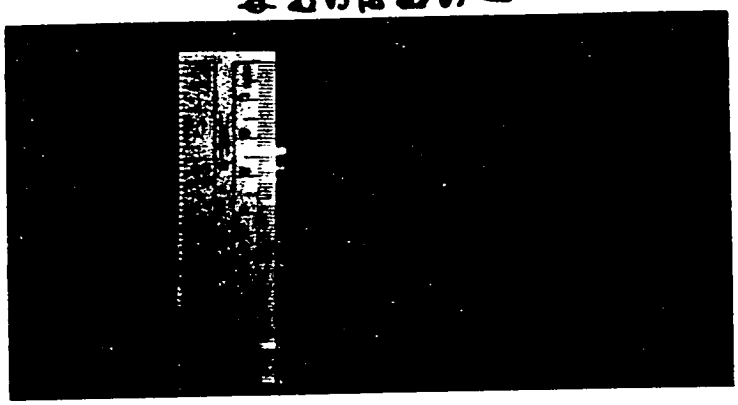

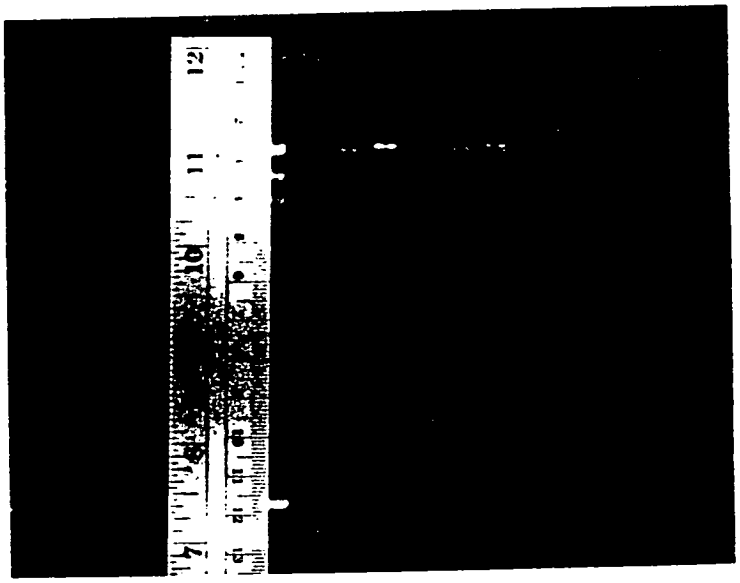

Extracting Eth. Brom. [redacted]/87

Added IsoAmyl Alcohol (5ml) to plasmid DNA taken of $CsCl_2$ density gradient
Shake vigorously for one minute
Spin at 3000 rpm for ~~5 min~~ 3 min
Repeated 3 X

DMD Clones

	[1]DNA	[2](µl)	[3]Buff	[4](µl)	[5]Enz	[6](µl)	[7]RNase A	[8]Spermidine	TE	Total
1	Marker	20								20
2	30.1(3)	5	E	2	EcoRI	2	2	1	3	15
3	30.1(4)	5	E	2	"	2	2	1	3	15
4	30.2(5)	5	E	2	"	2	2	1	3	15
5	30.2(6)	5	E	2	"	2	2	1	3	15
6	44.1(7)	5	E	2	"	2	2	1	3	15
7	44.1(8)	5	E	2	"	2	2	1	3	15
8	47.4(9)	5	E	2	"	2	2	1	3	15
9	47.4(10)	5	E	2	"	2	2	1	3	15
10	63.1(11)	5	E	2	"	2	2	1	3	15
11	63.1(12)	5	E	2	"	2	2	1	3	15
12	9.7 (1)	5	2, 1/2 1M NaCl	2,	Hind, Eco	1, 1	2	1	2	15
13	9.7 (2)	5	2, "	2,	Hind, Eco	1, 1	2	1	2	15
14										
15	5µl dye									
16	1½ hr, digest									
17										
18										
19										
20										
21										
22										
23										
24										
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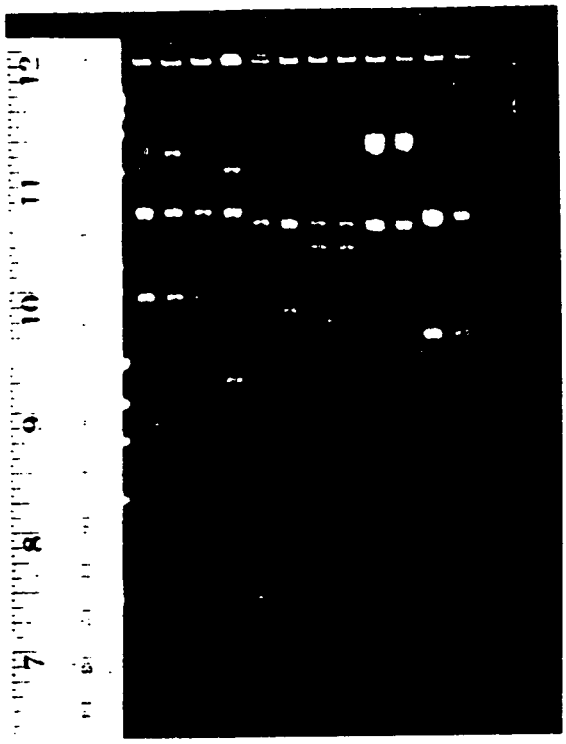
27										
28										
29										
30										
31										



Digest 1 hr pm

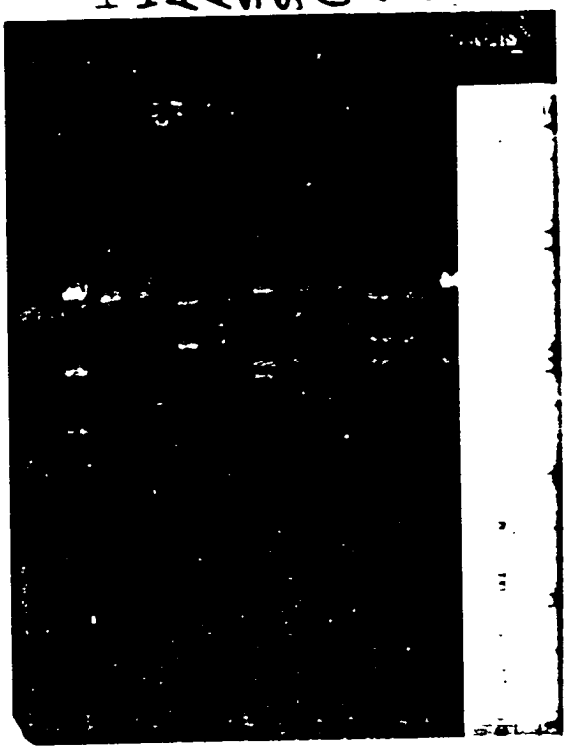
2nd Gel ☆ 12.1, 12.2 (10-1)

	1 DNA	2 (µl)	3 10xB	4 (µl)	5 Enz	6 (µl)	7 TE (µl)	8 Spermidine 2	RNase A 3
1	MARKER	20							
1	12.1	5	2	1.5	HindIII	5	23	2	3
2	12.2	5	2	1.5	HindIII	5	23	2	3
3	12.1	5	2	1.5	Asp718	5	23	2	3
4	12.2	5	2	1.5	Asp718	5	23	2	3
5	12.1	5	3	1.5	Sal+Bam	3+2	22	2	3
6	12.2	5	3	1.5	Sal+Bam	3+2	22	2	3
7	12.1	5	2,3	1.5	HindIII, Sal	2+2½ (40 / 25)	21.5	2	3
8	12.2	5	2,3	1.5	HindIII, Sal	2+2½ (40 / 25)	21.5	2	3
9	12.1	5	2,3	1.5	Asp, Sal	2.5+2.5	21	2	3
10	12.2	5	2,3	1.5	Asp, Sal	2.5+2.5	21	2	3
12									

10.1 x 13.8 cm
Sal 10units/µl
Bam 15 "/"
Hind 20 "/"
Asp718 12 "/µl (14mA)
Run o/n at 25V,
Gel size 11x14

L→R 12.1, 12.2,

HindIII
HindIII
Asp718
Asp718
Sal I+BamHI
Sal I+BamHI
(12.1) Hind+Sal
Asp718+Sal I
Asp718+Sal I



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Analysis of 12.1 and 12.2 by Restriction Digest

	DNA	*(µl)	10x Enzyme Buffer (µl)		Enzyme (µl)		*TE (µl)
1	Marker	20					
2	12.1	4.3	B*	5	BamHI	5	30.7
3	12.1	4.3	H	5	Sal I	5	30.7
4	12.1	4.3	E*	5	EcoRI	5	30.7
5	12.2	5	B*	5	BamHI	5	30
6	12.2	5	H	5	Sal I	5	30
7	12.2	5	E*	5	EcoRI	5	30
8							
9	Spermidine	2λ					
10	RNase A	3λ					
11							
12							
13							
14							
15							
16							
17							
18							
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21							
22							
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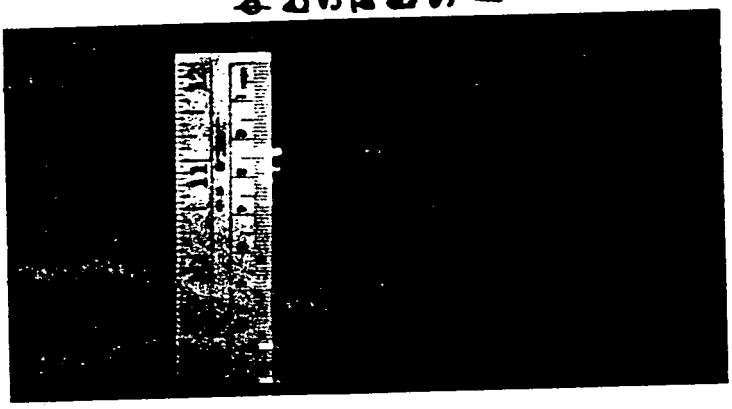
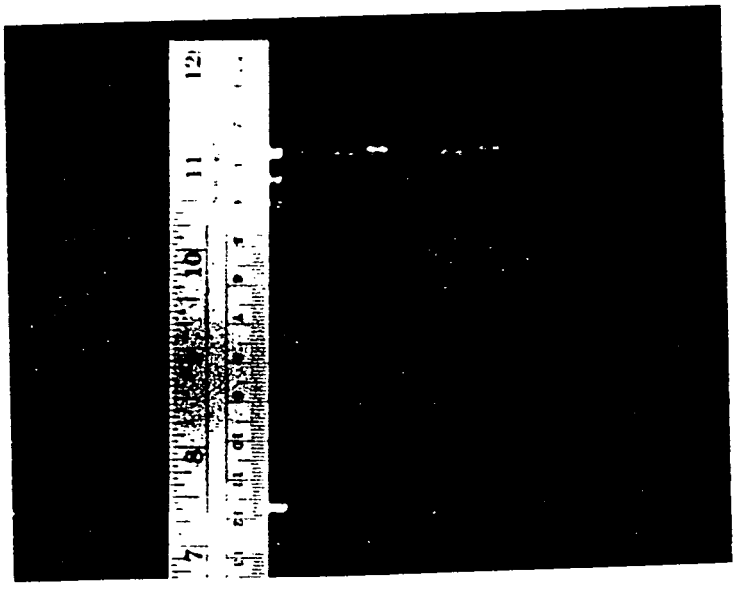
28							
29							
30							
31							

λ BamHI Sal I EcoRI BamHI Sal I EcoRI





Lifts of NT.1, NT.2, NT.3, 20 (Hu Gen Lib. vs XJ10) [redacted]/87

Phage eluted overnight 8/9
Picks (3°) taken from ~~[illegible]~~, NT.1, NT.2, NT.3 (certain pos)
Replated 2° NT.1, NT.3, 20
Lifts were made from

NT.1	NT3	NT.2	20
2°, 80λ	2°, 30λ	3°, 30λ	2°, 30λ
3°, 50λ	3°, 30λ		

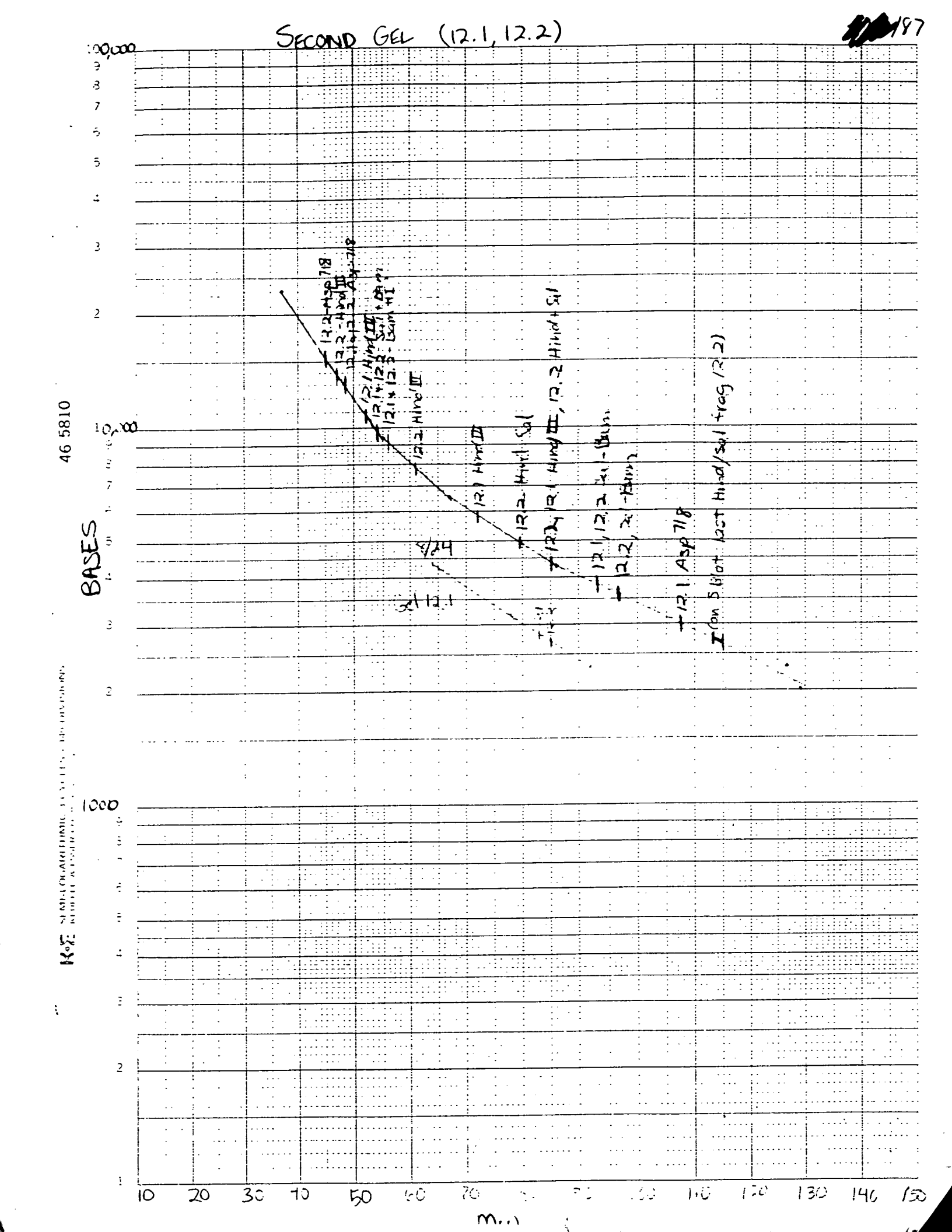
Needed to replate NT.1, 3°, 50λ → used 10λ
Gel run on 12.1, 12.2 phage lysate
- could have some protein contamination because phenol/chloroform extraction not shaken enough

Prehyb. 6 lifts above, NT.1, NT.3, NT2, 20
Hyb. - Added 6×10^6 cpm to 6 filters
XJ10 labelling (8/6/87) 20,000cpm/μl

Fragment Sizes of second Gel 12.1, 12.2

1	2	3	4	5	6	7	8	9
12.1	12.2	12.1	12.2	12.1	12.2	12.2	12.1	12.2
HindIII	HindIII	Asp718	Asp718	Sal+Bam	Sal+Bam	Hind Sal	Asp Sal	Asp Sal
				9.2	9.2			
10.8Kb	13.8Kb		15.3Kb	~~9.2Kb~~	~~9.7Kb~~	9.2Kb	12.9Kb	12.9Kb
5.85Kb	7.8Kb	12.9Kb	12.9Kb	~~9.1Kb~~	~~8.9.1Kb~~	4.95Kb	9.2Kb	9.2Kb
4.35Kb	4.35Kb	3Kb	(2.85)	3.8Kb	3.8Kb	4.3Kb	(3.0)	(2.85)
					3.6Kb	(2.7)		

Library constructed w/ EMBL3 → 44 kb
(takes up to 23Kb insert)


SECOND GEL (12.1, 12.2)
187
100,000
10,000
1000
BASES
46 5810
12.2 HindIII
12.2 HindIII Sal
12.2 HindIII, 12.2 HindIII Sal
12.1 / 12.2 Xba-Bam
12.2 Xba-Bam
12.1 Asp 718
Iron Blot last HindIII Sal trog (12.2)
10
20
30
40
50
60
70
130
140
150

[redacted]/87

Developed Autoradiogram ī 2° and 3° of Hu. Gen. Lib. vs. XTIO
Picked NT. 2, 3°, 30λ and NT. 3 3°, 30λ
Plated out 4° and titered - see next page
Blotted second gel 12.1 and 12.2
Washed, baked and washed first blot 12.1 and 12.2
Prehybed lift from less dense NT. 1 -10λ (3 ml 40% F
and first blot 12.1 + 12.2 (7.5 ml 50% F)

Titering 3° of NT.3 and NT.2

NT.2 30λ

39 x 33.33 = 1300/ml = 1.3/μl

NT.3 30λ

459 x 33.33 = 15,300/ml = 15.3/μl

1-78
2-57
3-69
4-116
5-69
6-80
459

*use 100λ of full strength ≅ 1500 pfu

Mini-Prep of DMD Clones

[redacted] 7

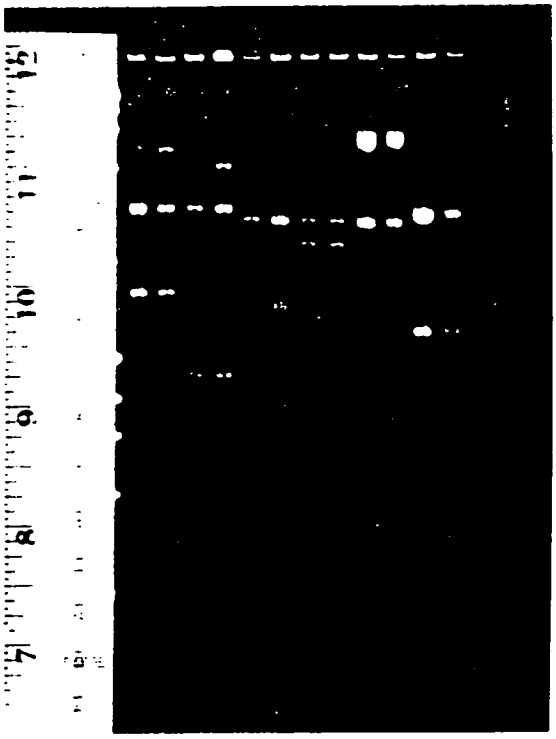
9.7a	1	
" b	2	
30.1a	3	
" b	4	
30.2a	5	
" b	6	
44.1a	7	
" b	8	
47.4a	9	
" b	10	
63.1a	11	
b	12	

To make soln. 2 Add H_2O 1st, SDS, NaOH
O/N culture, 2 picks from each clone
1 ml of gently vortexed culture into microfuge
Spun down bugs 15 sec
Aspirated supernatant
Resuspended in 100µl soln I by vortexing vigorously
Added 200µl soln. 2, inverted 6x, ice 5 min
Added 150µl soln 3, inverted 3x, ice 30 min
Vortex gently
Spun 4 min
Poured sup. into tubes containing 800µl EtOH each
-70, 15' (layer weren't well mixed, thawed, mixed, -70, 10'
Spun 4 min

DMD Clones

[redacted]/87

	1 DNA	2 (µl)	3 Buff	4 (µl)	5 Enz	6 (µl)	7 RNase A	Spermidine	TE	Tot
1	Marker	20								20
2	30.1(3)	5	E	2	EcoRI	2	2	1	3	15
3	30.1(4)	5	E	2	"	2	2	1	3	15
4	30.2(5)	5	E	2	"	2	2	1	3	15
5	30.2(6)	5	E	2	"	2	2	1	3	15
6	44.1(7)	5	E	2	"	2	2	1	3	15
7	44.1(8)	5	E	2	"	2	2	1	3	15
8	47.4(9)	5	E	2	"	2	2	1	3	15
9	47.4(10)	5	E	2	"	2	2	1	3	15
10	63.1(11)	5	E	2	"	2	2	1	3	15
11	63.1(12)	5	E	2	"	2	2	1	3	15
12	9.7(1)	5	2 + 1/2 1M NaCl	2	Hind, Eco	1, 1	2	1	2	15
13	9.7(2)	5	2, "	2,	Hind, Eco	1, 1	2	1	2	15
14										
15	5µl dye									
16	1½ hr. digest									
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										



[redacted]/87

Finished mini-prep of DMD cDNA
For hyb. of NT.1, 10λ used 5ml probe (XJ10), 60ml H.S.DN
Ran gel of DMD cDNA
Prehybed second gel of 12.1, 12.2
Both in refrig. over the weekend (First + Second gel of 12.1, 12.2)
Start Y1088 culture

For the weekend

Titer stock
Wash and expose NT.1 vs. XJ10
Size DNA fragments from
1) Characterization of XJ10
2) First gel 12.1, 12.2
3) Second gel 12.1, 12.2

[redacted]/7

Estimation of Amplified phage lysate titer

From previous plates titers were determined

NT.2 and NT.3

NT.2 1.25×10^4/ml (2500 on plate to elute) ÷ 5 ml (SM) = 6.25×10^6/ml
NT.3 1.5×10^4/ml (1500 ") ÷ 5 ml " = 4.5×10^6/ml

NT.2 50 ÷ 6.25×10^2 = 80 µl
NT.3 50 ÷ 4.5×10^2 = 110 µl

Plated out NT.2, $1/10^3$, 80λ
$1/10^4$, 80λ
NT.3, $1/10^3$, 110λ
$1/10^4$, 110λ

Titer of NT.2, NT.3

NT.3 110λ, $1/10^4$
1-42
2-55
3-64
4-73
5-95
329

$329 \times 9.1 \times 10^4$ = 3×10^7/ml

Plate out ~~10λ~~ 13λ of full strength for 400,000

NT.2 $1/10^4$, 80λ
1500

$1500 \times 12.5 \times 10^4$ = 1.9×10^8/ml

~~1/10 (10λ, 90λ) dil., 15.8 µl~~ 2.1λ

4/13/87

EFFICIENCY LINE 22-206

Started lg. scale plasmid prep. for DMD cDNA clones
Prepared TB - 500ml per flask
Added Amp to 50µg/ml
Added 3mls to each
ON shaker O/N

Picked from original 2° plates - NT. 1 B, 20

Titered amplified phage lysate for lg. scale isolation of the DNA.

Hybridization to 2 Southern Blots 12.1, 12.2

XD-1

- labelled to 65,000 counts/μl
- want 500,000 c/ml
- 7 ml. for Blot 1 → 54 μl
- 7.5 ml. for Blot 2 → 62 μl
  116 μl

H.S. DNA → 140 μl
" → 150 μl

- Started prep of phage lysate DNA NT.2 and NT.3
- Maxi prep of DMD clones spun o/N on $CsCl_2$ gradient

- phage lysate DNA - NT.2 OD_{260} = 0.39
NT.3 " = 0.35

NT.2 → 0.39 x 100 x 50 = 1.95 mg/ml
NT.3 → 0.35 x 100 x 50 = 1.75 mg/ml

For 10µg DNA

NT.2 → $\frac{10}{1950}$ = 5.1µl ~ 5λ

NT.3 → $\frac{10}{1750}$ = 5.7µl ~ 6λ

Washed Southerns on 12.1 + 12.2
2x5' 2xSSC RT
1x30' 2xSSC 65°C
1x30' 0.1xSSC 50°C

ON Film

Set up digestion of NT.2, NT.3
0.7% Gel
Hind III
EcoR I
Sal I
Eco, Sal
Hind, Sal

Hybridization - NT.1, 20 vs XJ10

[redacted]/87

Aprox. 2 weeks ago XJ10 → 20,000 counts/μl
now → 10,000 c/μl
Want 2×10^6 counts → take 200μl
Total vol. 5 ml.

$5x = (.1)5$
$x = .1$ ml HS DNA

Results seem inconclusive
Much of the probe was lost because the sealer would not seal
Washes - 2x, 2x SSC RT 5min
2x, 2x SSC 60°C 20'
1x, .1x SSC 45°C 15'

Set up Southern Blot of NT.2, NT.3

Completed ~~Miti~~ phage lysate DNA purification, resuspended in 1 ml

DNA removed from $CsCl_2$ gradient
EtBr removed by extraction ~~&~~ Isoamyl Alcohol
Added 2 vol. EtOH
Freezer (-20°C) O/N

Ran gel on NT.2, NT.3

Developed Autorad. of Southern Blots of 12.1, 12.2
[Washes were 1x 30' 2XSSC, 65°C, 1x 30' 0.1xSSC] 50°C

NH_4Ac Precipitation to Further Purify [redacted]/87

DMD cDNA (in 95% EtOH)
- removed from -20°C, centrifuged
- $CsCl_2$ present
- Washed w/ 70% EtOH
- Resuspended in 1ml TE
- Heated at 37°C until $CsCl_2$ in soln.
- Transfered to new tube
- Added 50µl 5M NaCl (vor.)
- Added .5ml 7.5M NH_4Ac (vor.)
- 3ml 95% EtOH - ice 30'
- Spin 12K 10'
- 2ml 70% EtOH
- 0.5 TE in microfuge tube

12.1 12.2 (in 0.5ml TE)
- Add ½ vol. 7.5M NH_4Ac (vor.) 250µl
- Ice ~~30'~~ 5'
- Microfuge 10'
- Transfer to blue tubes 1.5ml EtOH
- 30' ice
- Spin 12K 10'
- Rinse 70% EtOH
- Resuspend 0.5ml TE

Washed S. Blot
2x NaOH 10'
2x Neut. 10'
~~Rinsed~~ filter (hydrated) ∈ H_2O, 20x SSC
Transferred in 20x SSC
10.1 x 13.9 cm

12.2 Preparatory Gel

[redacted]/17

	1 DNA	2 (µl)	3 10xB	4 (µl)	5 Enz	6 (µl)	7 H_2O (µl)	8 Spermidine	9 RNase A
1	λ-ØX	30							
2	B12.2	250	2	40	HindIII	20	50	20	20
3				+44λ 5M NaCl	+SalI (15U/λ)	25			
4									
5									
6	0.7% Gel								
7	vol.								
8	2½ hrs. then 2nd Enz. 2 hrs								
9	NaCl?								
10	Enz.								
11									
12	This gel sample was run on bg gel								
13	with DMD cDNA inserts								
14									
15									
16	Small 12.2 fragment cut ε Hind, Sal seems								
17	to be								
18									
19	2.75 Kb								
20									
21	picture w/ Gel picture next page								
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Conc. of DMD DNAs

	1	2	3	4	5	6	7	8	9
1		1/500 dilution							
2	DNA	260 ↓	260/280	conc.	vol.	yield			
3	9.7	.479	2.00	4.0 ng/λ	½ ml	2 mg			
4	30.1	.433	2.00	3.6 ng/λ		1.8 mg			
5	30.2	.631	1.97	5.3 ng/λ		2.6 mg			
6	44.1	.325	1.98	2.7 ng/λ		1.35 mg			
7	47.4	.290	1.99	2.4 ng/λ		1.2 mg			
8	63.1	.182	2.00	1.5 ng/λ		760 mg			
9	12.1	.171	2.28	1.4 ng/λ		700 mg			
10	12.2	.132	2.30	1.1 ng/λ	↓	550 mg			
11									
12	12.2i			4 ng/λ (9λ remaining)					
13	9.7i			20 ng/λ					
14	30.1i			20 ng/λ					
15	30.2i			20 ng/λ					
16	44.1i			23 ng/λ					
17	47.4i			8 ng/λ					
18	63.1i			15 ng/λ					
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

12.2 Preparatory Gel

[redacted]/17

1 DNA	2 (µl)	3 10xB	4 (µl)	5 Enz	6 (µl)	H2O (µl)	Spermidine	RNase A
λ-ØX	30							
B12.2	250	2	40	HindIII	20	50	20	20	400
			+4.4λ 5M NaCl	+SalI (15U/λ)	25				

0.7% Gel
vol.
2½ hrs. then 2nd Enz. 2 hrs
NaCl?
Enz.

This gel sample was run on lg. gel with DMD cDNA inserts

Small 12.2 fragment cut ε Hind, Sal seems to be

2.75 Kb

picture w/ Gel picture next page

EFFICIENCY LINE · 22-206 AMPAD

Gel ε 12.1, 12.2 (3rd gel) to see if 2.75 frags Hind III frag)

	DNA	(µl)	10xB	(µl)	Enz	(µl)	TE (µl)	Spermidine	RNAse A (µl)
2	12.1	7	2	5	Hind III	5	28	2	3
3	12.2	9	2	5	Hind III	5	26	2	3
4	12.1	7	2, 1M NaCl	5, 2.4	Hind, Sal	2, 3	28	2	3
5	12.2	9	2, 1M NaCl	5, 2.4	Hind, Sal	2, 3	26	2	3
1	λ-Px	20							

EFFICIENCY LINE • 22-206

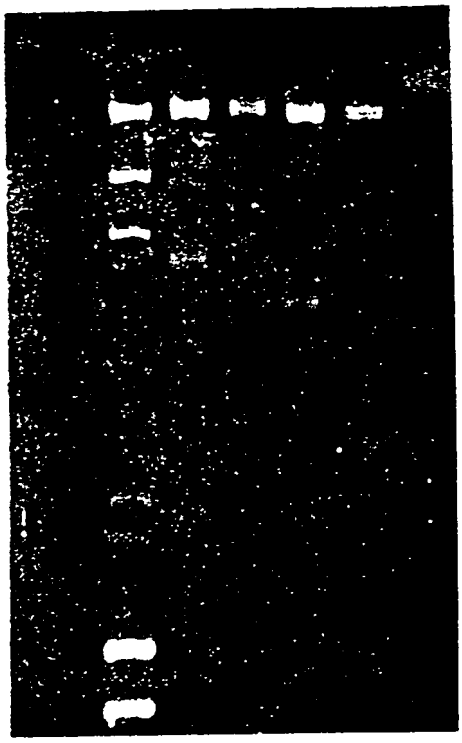
For double digests - added 2nd Enz. after 2.5 hrs. then 2 hrs.

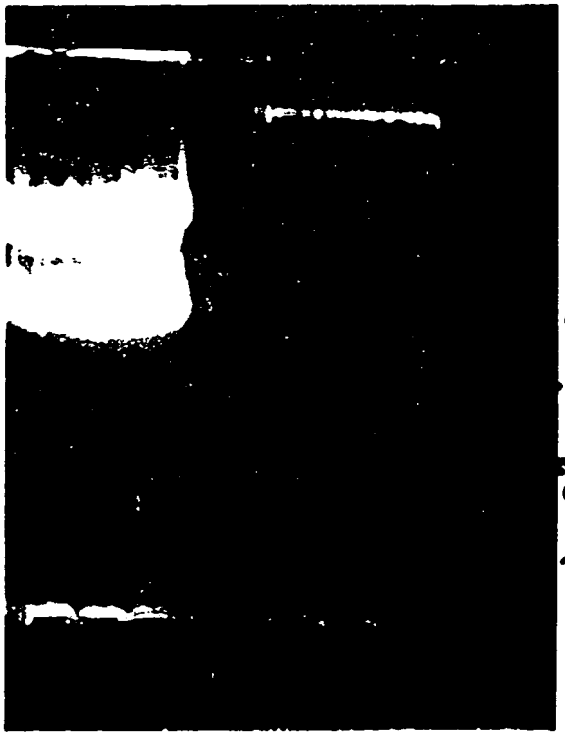
new conc.

12.1 1.4 mg/ml

12.2 1.1 mg/ml

From Prep Gel 12.2





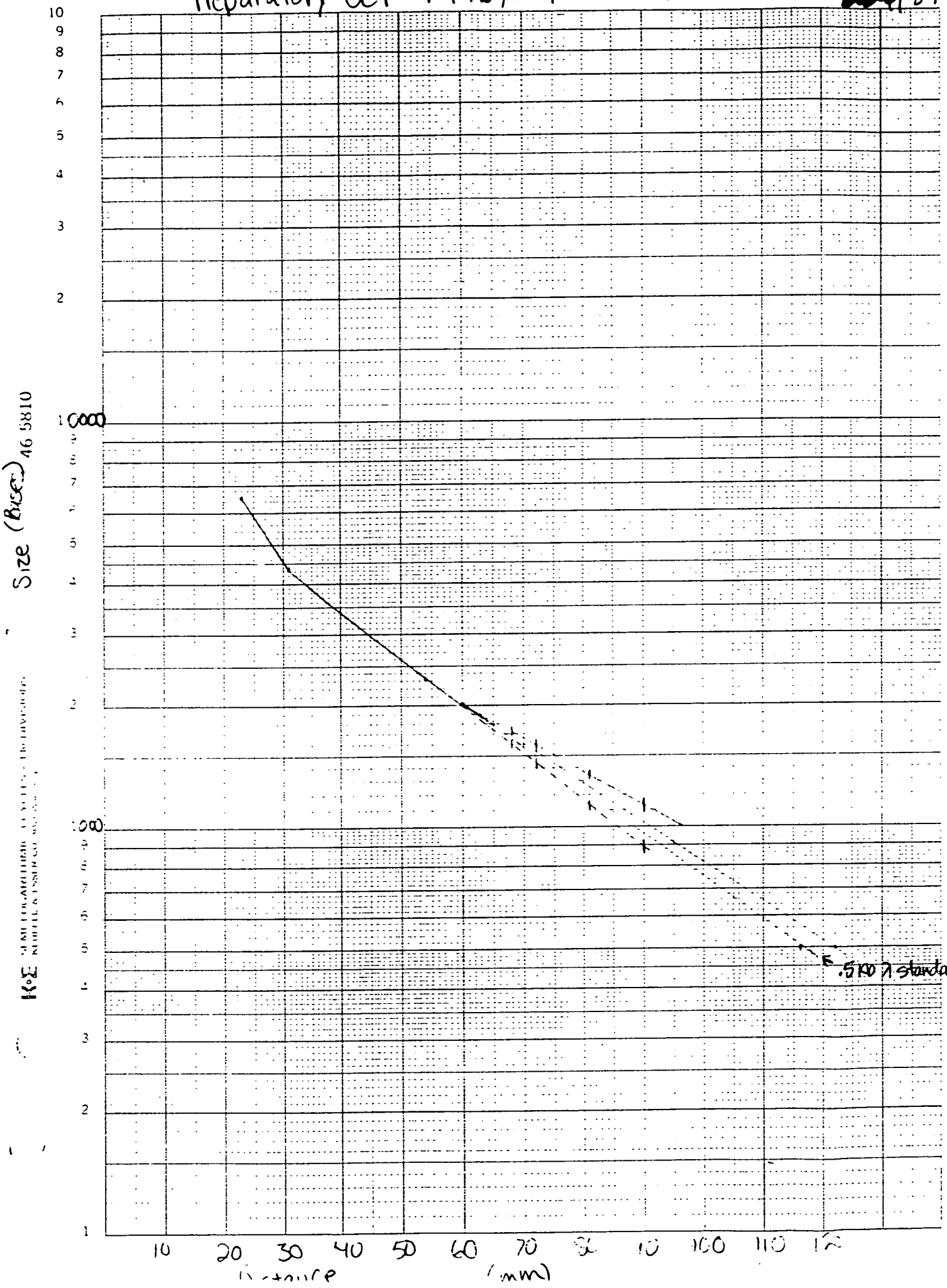
2.75 2.4

Sized quickly on graph 8/11

ISOLATED DMD INSERTS

Clone	Size	RE Sites	Concentration	Volume
12.2	2.75kb	HindIII/Sal I	4ng/µl	10µl
NT.1b	4.30kb	HindIII	18ng/µl	10µl
NT.2	3.35kb	HindIII/Sal I	30ng/µl	10µl
NT.3	3.20kb	EcoRI/Sal I	18ng/µl	10µl
20	1.25kb	HindIII/Sal I	8ng/µl	10µl
PTZ18R	2.90kb	Sal I	50ng/µl	48µl
PTZ19R	2.90kb	HindIII	50ng/µl	80µl
47.4	0.60kb	EcoRI/Bgl II	20ng/µl	30µl
63.1	1.00kb	HindIII	40ng/µl	30µl

Preparatory Gel NT.16, 20, PTZ18R, PTZ19R



Approx. Sizes of lower Bands 20 (H3/sal) [redacted]/87

	Range	Average
1	1.6–1.72Kb	1.66Kb
2	1.45–1.6Kb	1.52Kb
3	1.13–1.35Kb	1.24Kb ← to electrolute
4	0.89–1.14Kb	1.02Kb

Fragment on gel of NT, 16 and 20 at 128mm in lane containing clone 20 is 1.25Kb

Saved

20 H3/Sal frags. 1.66Kb
1.52Kb
1.02Kb

EFFICIENCY LINE · 22-206

For prep gel NT.2, 3, 63, 47.4

NT 2 Hi-dIII-Sac
3.3kb (3.35) ✓
1.8kb
(4.3-4.5 λ doublet)

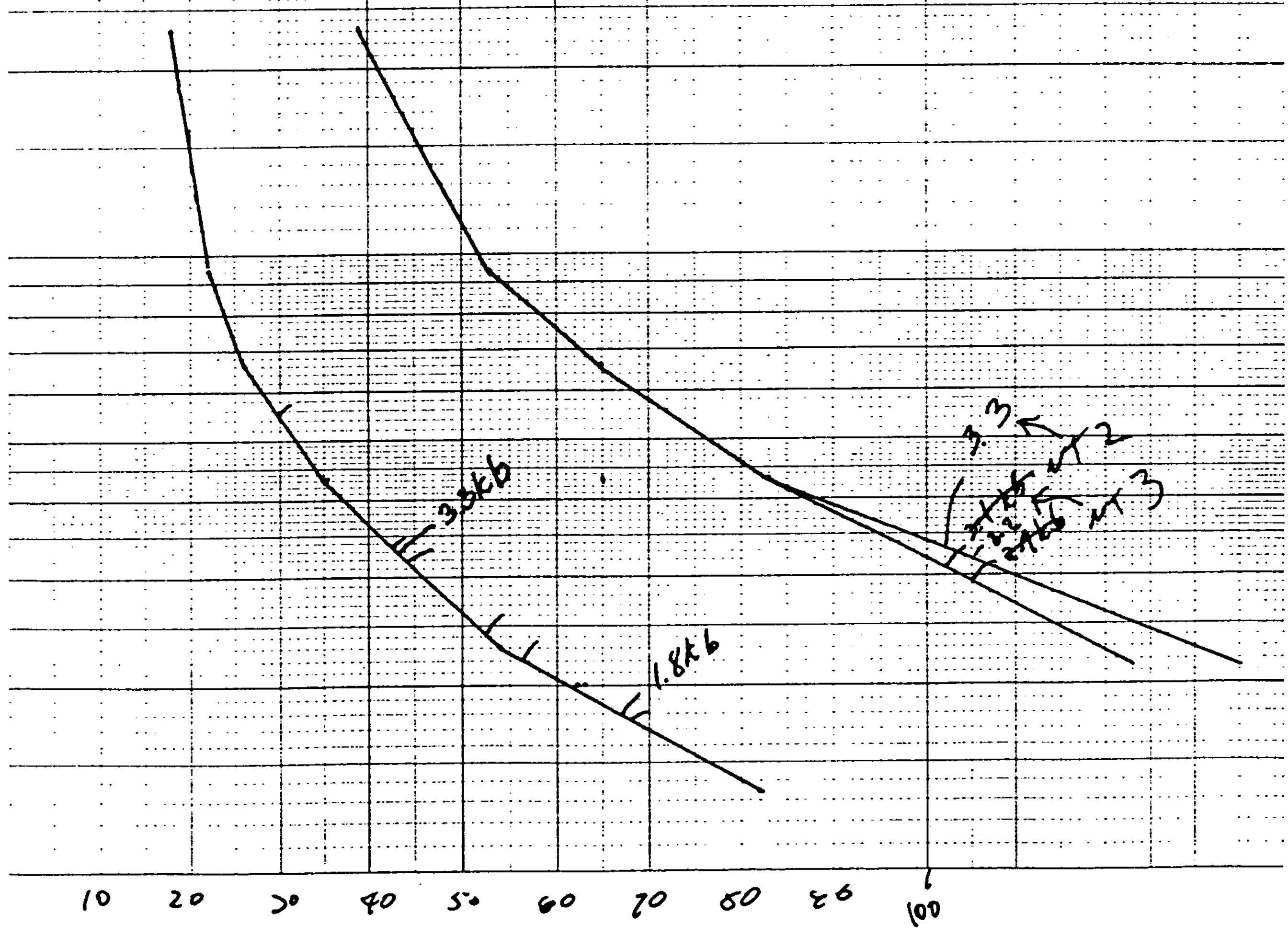
18
22
26
35
54
63
82

N.T. 3 Eco-Sal
5.5kb
3.2kb ✓
2.4kb
2.2kb
1.75kb

3.9
53
65
83

46 5810

K+E SEMI-LOGARITHMIC 2 CYCLES × 140 DIVISIONS



EFFICIENCY LINE · 22·206

$\frac{14 \text{ days}}{2 \text{ (double)}} = \frac{11 \text{ days}}{X}$

$X = 1.57$ (x 133% if probe fresh)

probe to be used = 210%

H.S. DNA = 140%

Recounted probe

F_1 2.9×10^5 cpm 2.9×10^3/x

F_2 2.3×10^6 cpm 2.3×10^3/x

Take 2600 x of F_2 for 6×10^6 cpm counts

Combined remaining probe

Added all

F_1 122 T=002.00 A=026282.0(1.0%) B=001103.5(5.0%) C=000000.0(>20%) S=0.168

F_2 122 T=002.00 A=024575.5(1.0%) B=003521.0(3.0%) C=000000.0(>20%) S=0.166

EFFICIENCY LINE® 22-206

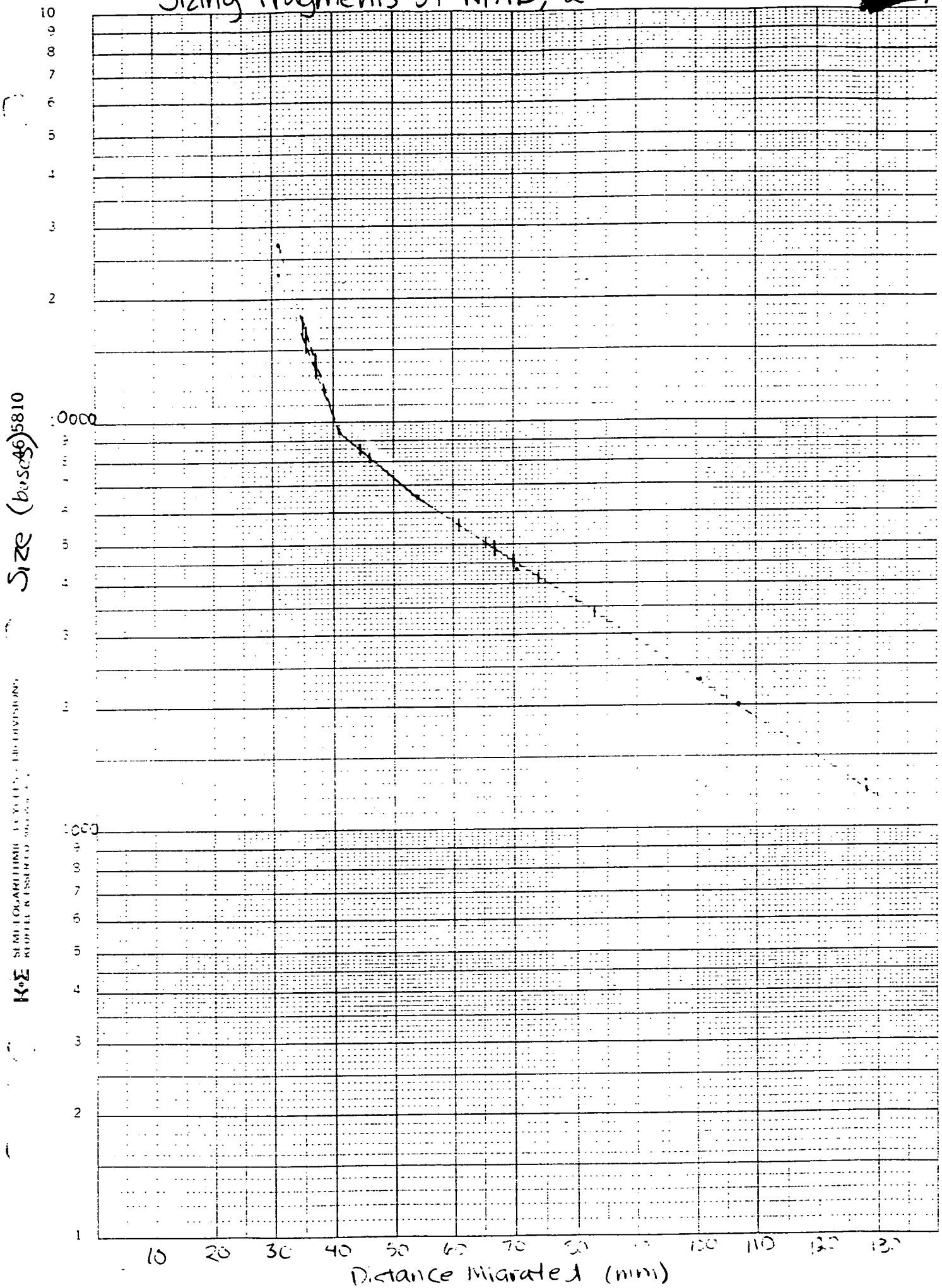
	A_{260}	conc.	vol.	yield
NT.1B	0.233	2.33mg/ml	0.5ml	1.17mg
NT.2	0.382	3.82mg/ml	"	1.91mg
NT.3	0.329	3.29mg/ml	"	1.64mg
20	0.230	2.30mg/ml	"	1.15mg

.147

For 30µg of ~~NT.2 → 8µl~~
~~NT.3 → 9µl~~
63.1 → 20µl
47.4 → 12µl

Sizing Fragments of NT.1B, 20

[redacted]87



(con't) [redacted] 87 [redacted] 87

	1 DNA	2 LANE	3	DISTANCE MIGRATED		7	8 SIZE	9
1	Enzyme							
2								
3	NT.1B	9		46.3			8.20	
4	Hind/Sal			61.0			5.60	
5				65.0			5.10	
6				69.5	70.0		4.55	
7								
8	20	10		37.0		~	13.80	
9	Hind/Sal			61.0			5.60	
10				65.0			5.10	
11				128.0			1.25	
12								
13	STANDARD							
14	3			54.0			6.55	
15	2			41.0			9.41	
16	~~3~~ 4			70.5			4.36	
17	~~4~~ 5			100.5			2.32	
18	~~5~~ 6			107.0			2.02	
19	~~6~~ 1 - ΦX			128.0			1.35	
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

EFFICIENCY LINE® 22-206

Purification of DMD clones NT.1B, 20

[redacted]/87

- 14ml. eluted from 150mm plates
- Blue tubes leaked a little when phenol/chlor. extracting
- Left in 95% EtOH O/N
- 15' -70°C 9/1/87
- Cfg 15' 11k, 4°C
- Wash 70% EtOH, Tubes collapsed- no leakage
- Dry
- Resuspend 0.5ml

NT.1B 5λ → 0.5ml

OD_{260} = 0.38 x

$0.38 \times 200 \div 20 = 3.8 \mu g/\mu l$

$30 \mu g \cong 8.0 \mu l$

20 5λ → 0.5ml

OD_{260} = 0.35

$0.35 \times 200 \div 20 = 3.5 \mu g/\mu l$

$30 \mu g \cong 8.5 \mu l$

EFFICIENCY LINE · 22-206

NT.1B 1/10⁴, 3.6λ

1-54
2-31
3-36
4-33
154

154 × 277.78 = 4.2 × 10⁴ × 10⁴ = 4.2 × 10⁸/ml

20 1/10⁴, 10λ

2000 × 100 × 10⁴ = 2.0 × 10⁹/ml

NT.1B - 400,000 ÷ 4.2 × 10⁸ = 1 µl
Do 1/10 dil. take 10 µl

20 - 400,000 ÷ 2.0 × 10⁹ 20
Do 1/100 dil. take 10.7 µl

Screening NT.1B, 20 4° Lifts

[redacted]/7

- XJ10 labelled to 45,000 counts/μl (F, labelling 8/24,
- 5ml at 1×10^6 counts/ml
- $\frac{5 \times 10^6}{4.5 \times 10^4} = 110\,\mu l$
- For 0.1mg/ml HS DNA
  $5x = (0.1)5ml$
  $x = 100\lambda$

EFFICIENCY LINE® 22-206

Titering NT.1B, 20 Amplified phage lysate

From 8/27 titers were NT.1B = 84.2 pfu/μl, 8.4×10^4/m
20 = 51 pfu/μl, 5.1×10^4/m

NT.1B 100λ

$(8400 \times 10 =) 8.4 \times 10^4$/ml
8.4×10^4/ml (8400 on plate) ÷ 5ml = 1.4×10^8/ml

20 100λ

5.1×10^4/ml (5100) ÷ 5 = 5.2×10^7/ml

50 ÷ 1.4×10^3 = 36μl NT.1B
50 ÷ 5.2×10^3 = 9.6μl 20

20
- 1/100 → 5.2×10^5 [illegible]
  1/100 → 5.2×10^3 → [illegible]λ, 100λ

NT.1B
1/100 → 1.4×10^6
1/100 → 1.4×10^4 → 3.6λ, 36λ

Titering 20 and NT.1B For Amplification [redacted]

20 5λ 4°

1-90
2-52
3-48
4-65

255 x 200 = 51/μl
1.5x10³/51 = 30λ of a pick from 4° plate

NT.1B 5λ 4°

1-45
2-162
3-65
4-99
421

421 x 200 = 84.2/μl
1.5x10³/84.2 = 20λ of a pick from 4° plate

EFFICIENCY LINE® 22-206

Ethanol Precip. of Electroluted DMD cDNA [redacted]/7

- Samples removed from -20°C
- Placed 15-30' at -70°C
  " 37°C 5' (to get agar from electrolution heated)
- Centrifuged horizontal microfg. 12'
  " angle " 10'
- Removed supernatant carefully
- Added 200λ 70% ethanol
- Centrifuged in angle in cold room 5'
- Removed supernatant
- Speed vac 5'
- dissolved in 50λ TE
- ~~R~~

Washing Filters NT.1, .1B, NT.20, NT.3 [redacted]/7

S. Blot of NT.2, .3

For small filters:

2X 2xSSC RT 5'
2X 2xSSC 65°C 20'
1X 0.1xSSC 45°C ~~20~~ 15'

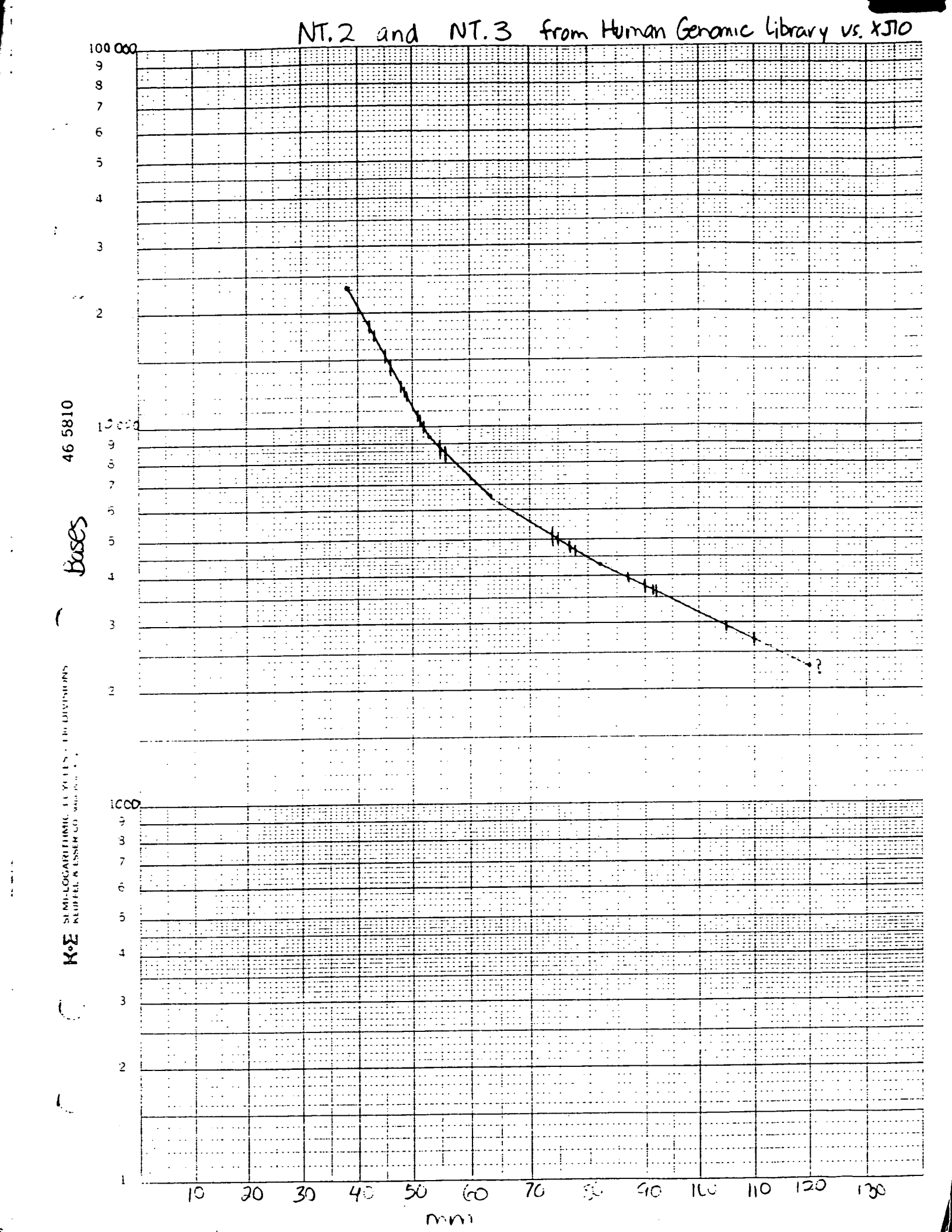
For S. Blot of NT.2, NT.3

2x 2xSSC 5' RT
1x 2xSSC 30' 65°C
1x 0.1xSSC 30' 45°C – better at 50°C
(less background
Comparing S Blot 12/12...
to results from here

<u>Results of Autorads</u> 8/26/7

- 3 Picks from NT.1B, 20 appear positive
- Will pick again and titer tonight

- Even though S.Blot autorad. went O/N
looks good
no internal HindIII sites


NT.2 and NT.3 from Human Genomic Library vs. XJ10
100 000
9
8
7
6
5
4
3
2
1 000
9
8
7
6
5
4
3
2
Bases
46 5810
1000
9
8
7
6
5
4
3
2
1
10
20
30
40
50
60
70
110
120
130
mm
?

	[1]DNA	[2]Lane	[3] Distance migrated				Size	Fragment	
1	NT.2	7	530	mm			9.41	Kb-λ	
2	Eco,Sal		~~55.5~~	mm		13.4	8.70	Kb	
3			~~58.5?~~ ~~78.0~~	mm			4.70	Kb-λ	?
4			82.0 ~~82.0?~~	mm			4.36	Kb-λ	?
5	NT.2	8	~~53~~.0	mm			9.41	Kb-λ	
6	Eco,Sal		~~56~~.0	mm			~~8~~.50	Kb	
7			~~74~~.0	mm			5.10	Kb	
8			49.0? ~~43.0~~ ~~46.0~~	mm			17.50	Kb	
9	NT.2	9	49.0	mm			14.50 11.00	Kb Kb	
10	Hind,Sal		53.0	mm			9.41	Kb-λ	
11			78.0	mm			4.70	Kb-λ	
12			82.0	mm			4.36	Kb-λ	
13			92.0	mm (faint)?			3.63	Kb-	
14	NT.3	10	49.0				11.00	Kb	
15	Hind,Sal		53.0				9.41	Kb-λ	
16			~~74~~.0?				5.10	Kb	
17			78.0				4.70	Kb-λ	
18			82.0				4.36	Kb-λ	
19									
20	NT.2	9	~~105~~.0				2.95	Kb	
21	(autorad)								
22	NT.2	9	~~110~~.0				2.70	Kb	
23	NT.3	10	~~91~~.0				3.95	Kb	*
24									
25									
26									
27									
28									
29									
30									
31									

Gel Distances NT.2, NT.3

[redacted] 7

	1	2	3	4	5	6	7	8	9
1									
2	λ-Hind III		Distance migrated			Size fragment			
3	1		38 mm			23.13 Kb			
4	2		52 mm			9.41 Kb			
5	3		63.5 mm			6.55 Kb			
6	4		82 mm			4.36 Kb			
7	5?		~420 mm			2.32 Kb			
8									
9	DNA	Lane	Distance migrated			Size fragment			
10									
11	NT.2	1	48.5 mm			12.30 Kb			
12	Hind III		63.5 mm			6.55 Kb - λ			
13			75.0 mm			5.0 Kb			
14			82.0 mm			4.36 Kb - λ			
15	NT.3	2	45.0 mm			15.30 Kb			
16	Hind III		✓51.5 mm			10.40 Kb			
17			155.0 mm			8.80 Kb			
18			77.0 mm			4.80 Kb			
19			82.0 mm			4.36 Kb - λ			
20	NT.2	3	51.0 mm			10.60 Kb			
21	EcoRI		82.0 mm			4.36 Kb - λ			
22			91.5 mm			3.68 Kb			
23	NT.3	4	✓46.0 mm			14.5 Kb			
24	EcoRI		56.0 mm			8.5 Kb			
25			90.0 mm			3.75 Kb			
26	NT.2	5	✓42.0 mm			18.5 Kb			
27	Sal I		52.0 mm			10.00 Kb			
28	NT.3	6	48.0 mm			12.80 Kb			
29	Sal I		53.0 mm			9.41 Kb - λ			
30			✓74.0 mm			5.1 Kb			
31									

Electroelution of DMD cDNA Inserts

Let slices sit in H_2O, (13 µl/1ml) - inverting every 5' or so (to rel
Set up cups w/ Dialysis Membrane
Filled w/ H_2O to check for leaks
Emptied, decanted H_2O from orange capped tubes (15
Removed slices, blotted dry ~~c~~ Kimwipe
Cut into small cubes on parafilm, wiping razor after each sample
Pieces placed in lg. well - loaded onto electroelution box
Allowed to elute for ~2hrs.

Jeff removed DNA.

Hybridization of XJ10 to NT.1, NT.3, 20

[redacted]

$\frac{9 \times 10^6 \text{ counts}}{45{,}000 \text{ counts}/\mu l}$ for 9 ml. = 200 µl
180 µl H.S. DNA

For Blot of NT.2, NT.3

$\frac{6 \times 10^6}{45{,}000}$ for 7 ml = 133 µl
140 µl H.S. DNA

Total
333 µl F1 probe
320 µl H.S. DNA

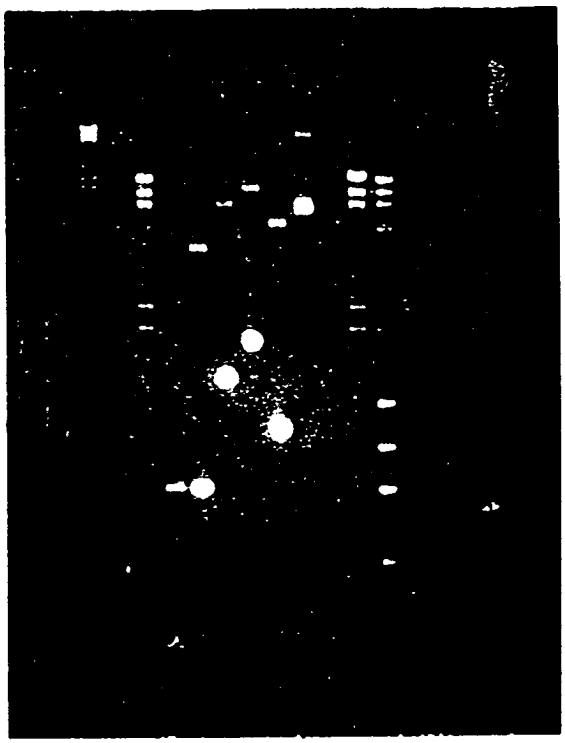
[redacted]

[redacted]

Second Gel to Determine Conc. of DMD cDNA's [redacted]

- Dilutions made of 5 clones (1/10) using 10x
- Run on same gel (12 aliquots)

-2	-1	3	4	5	6	7
new λ-HindIII	47.4	44.1	9.7	30.1	30.2	63.1



	conc	conc
9.7	20ng/µl	
30.2	35ng/µl	20
30.1	30ng/µl	20
47.4	8ng/µl	
44.1	23ng/µl	
63.1	15ng/µl	

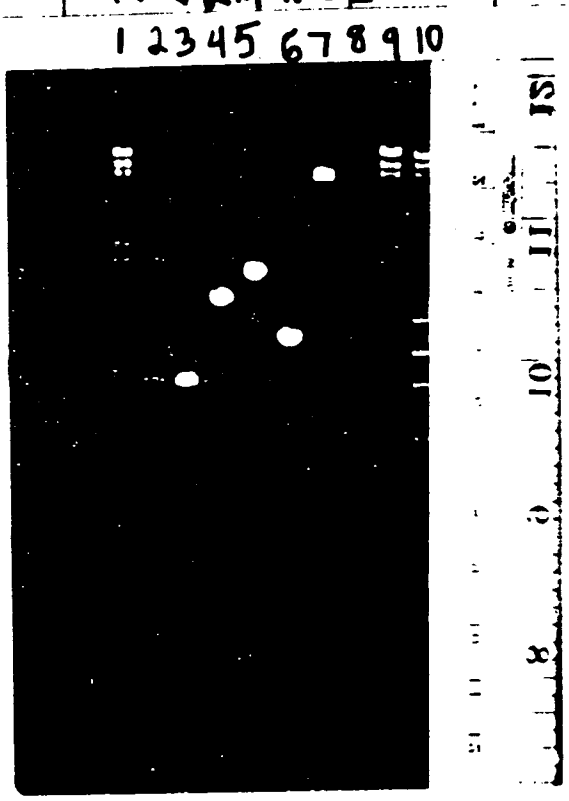
* dilute 30.2 & 30.1 to 20ng/µl = TE

Concentrations range from 20ng (47.4) to 35ng/µl (30.2)

Gel Run to Determine DMD cDNAs' Concentration (inserts)

- Aliquots were diluted from 50λ to 100λ
- 1λ of each was run on a gel with Jeff's λ-Hind III standard.

1	2	3	4	5	6	7	8	9	10
λ-Hind III 5λ	44.1 (before centrifuge) Hole in well	44.1 (after cfg.)	9.7	30.1	30.2	63.1	12.2 some missed the well	λ-H 5λ	λ-H 6λ



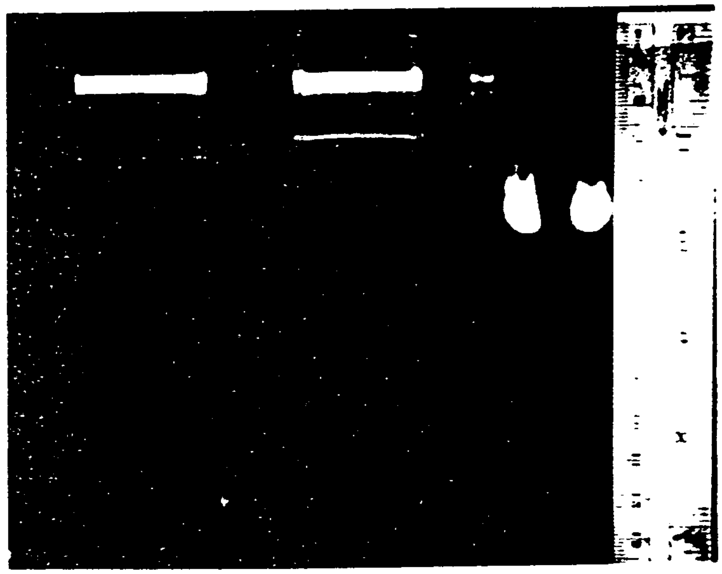
Concentration estimated to be 200-300ng/λ in 5 DMD cDNA's

Isolated Inserts [redacted]

	clone	2	size	4	RE's	6	7	8	conc.	VO
	12.2		2.75Kb		Hind/Sal				4ng/λ	10
	NT.2		3.35Kb		Hind/Sal		} run ¼ on gel		30ng/λ	10
	NT.3		3.2Kb		Eco/Sal		}		18ng/λ	10
	47.4		0.6Kb		Eco/Bgl II 3'		} dilute to 20λ		~50ng/	
							run ½λ on gel		(~20~~0~~ng/ 1:10 2λ	0.1.C
	63.1		1.0Kb		Hind		}			
	NT 16		4.3 kb		HindIII		} [illegible] ~ 20		18ng/λ	10
	λ20		~~[illegible]~~ 1.25Kb		HindIII-Sal		}		8ng/λ	10
Add 28λ	PT218R		2.9		~~HindIII~~ Sal I				120ng/λ	20
" 60λ ↳ TE	PT219R		"		~~Sal I~~ HindIII				200ng/λ	20
	reprecipitated (Phenol extracted)									
	47.4		0.6Kb		Eco/Bgl II 3'				20ng/λ	30
	63.1		1.0Kb		Hind				40ng/λ	30λ

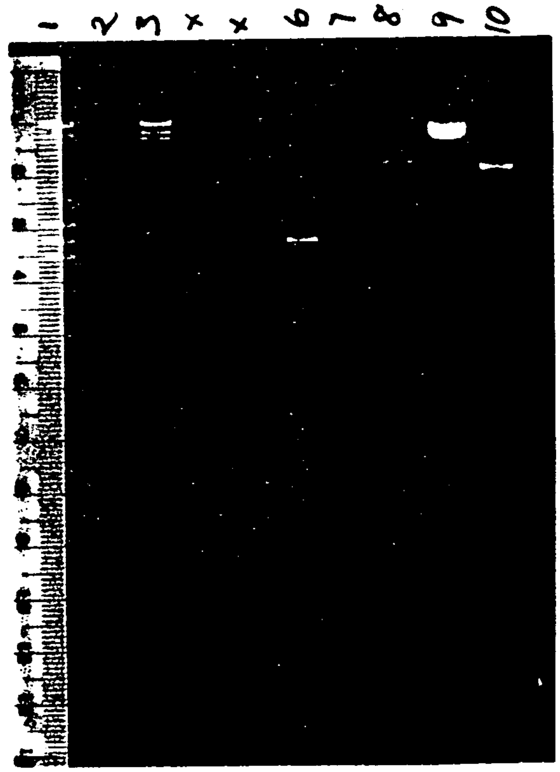
Preparation of Vectors for Subcloning

DNA	(µl)	10xBuff	(µl)	Enzyme	(µl)	Spermidine	RNAse A	H_2O	Tot
PTZ19R	5	2	5	HindIII	5	2	2	3	50
PTZ18R	5	10	5	Sal I	5	2	2	3	50



DNA Quantitation

1 My Standard
2 NT.3i 1.0λ of 10λ
3 Jeff's Standard
4 λ
5 λ
6 63.1i diluted to 20λ, ½λ run (1.900 HB)
7 47.4i (.6kb EBg)
8 NT.2i 1.0λ of 10λ
9 Jeff pLESACS insert
10 Jeff pTZ EcoRI ~100ng/λ?



Gel to Quantitate NT.1, 20, PTZ18R, 19R

[redacted]

	1 Lane	3	4	5	6
1	1	My standard			
2	3	NT.1b insert			
3	5	20 insert			
4	7	PTZ18R ~~Hind III~~			Sal I
5	9	PTZ19R ~~Sal I~~			Hind III
6	11	Jeff's standard			
7					
8			NT.1b	20	PTZ18R
9					PTZ19R
10	~150 x 250				

[redacted]

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Klenow Fill-in RXN c NT.2,3,20,12.2* [redacted]87

	NT.2,3 (μl)	20 (μl)
DNA	5	9
dNTPs	1	1
10X Klenow Buffer	1.5	1.5
Klenow	1	1
H_2O	7.5	2.5
	15λ	15λ

1 hr. 37°C
½ vol NH_4Ac
2 vol 95% EtOH

* viscous when pipetted
* Will directly ligate NT.1b

9/11/87

Jeff decided to phenol extract, EtOH precip.
Will redo Klenow fill-in

Dissolved in 11 μl H_2O

H_2O+DNA	11
dNTPs	1
10x Buff	1.5
Klenow	1.5
	15 μl

37°C 1 hr.
EtOH O/N

TCA Precipitation of 47.4, XDSA Labelling [redacted]7

After oligolabelling add 50λ .2× SET
Mix
~~Vortex~~ Remove 1λ from each rxn. - Add to 6λ HS DNA
Add ~ ½ mls 10% TCA
Vortex
Ice 5'
Filter thru GF/C filter
Rinse 3-4 times c 10% TCA
Rinse c 3 mls 95% EtOH
Dry (air)
Count in thin scint. vials c filter in toluene

9/10 Unsuccessful - due to use of α-P^{32} CTP instead of ATP
9/11 " - CTP instead of dCTP

Repeat O/N

Ligation of NT.1b, 2, 3, 20, 12 into pTZ

[redacted] /87

Centrifuged DNA from Blunt-end rxn.
Added 900µl 70% EtOH
Resuspended in 6½λ H_2O

For Blunt-end Ligations

DNA (+ H_2O)	6.5λ
10x Ligase Buff	1λ ← in 'digobox'
10mM ATP	1λ
T4 Ligase	1λ → ē restriction enzymes
vector	½λ → HincII cut pTZ, Box 7, 6th
	10λ

O/N R.T.

For HindIII Ligation NT.1b insert

DNA + H_2O	5λ
10x Ligase Buff	1λ
10mM ATP	1λ
T4 Ligase	½λ
pTZ19R-HindIII	1λ (50ng/λ)
H_2O	1½λ
	10λ

Mix gently (like ē Klenow)

O/N 4° - cold room

Transformation of DH5α F' [redacted]/87

IPTG 10%
XGAL 25%

Removed tube of (.5ml) DH5αF's - thawed on ice
Transfered 2μl to 5 tubes * pre-cooled
Added 2μl of a 1/10 dilution of Ligations
Gently Pipetted up and down
Aliquotted 100μl to microfuge tubes → -70°C (quick fr in fros
Let sit on ice 30 min.
Heat shocked 410sec 42°C
On ice
Added 80μl S.O.C.
Incubated 1 hr. 225 r.p.m.
Plated out ē 10% IPTG, 25% XGAL, all samples on Amp plates

All had positives except NT.3

* should have gently flicked to mix

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Concentration Determination of Phenol extracted

	1	2	3	4	5	6	7	8	9	
1			47.4, 0.6Kb Eco/Bgl							
2			63.1, 1.0Kb H3							
3										
4										
5										
6							47.4	0.6Kb	Eco/Bgl	20ng
7										
8							63.1	1.0Kb	H3	40ng
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
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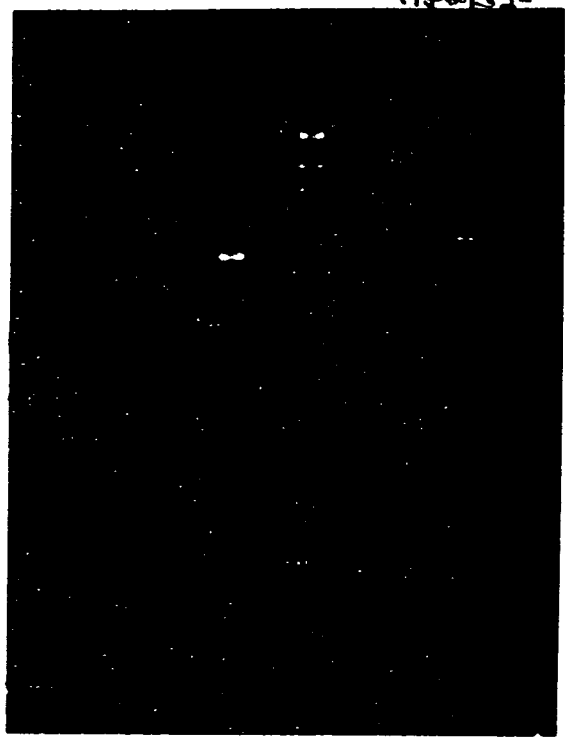
28										
29										
30										
31										



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Hybridization of Hu Gen. Lib. vs. 47.4 0.6 Kb E/Bg Fragment

[redacted]/87

Pre-hyb. - 80 mls → No Dextran Sulfate
H.S. DNA

Hyb. - 200 µl probe + 1.6 mls H.S. DNA
(~23 x 10^6 counts)

TCA precip. - Let go O/N
Repeated TCA precip.

* → **Results Negative** for screening of filters $\bar{c}$ 47.4 - 0.6 Kb E/Bg (lifted @ 7/16/87)

9/14 [illegible] A=351954.5(0.3%) B=351915.8(0.3%) C=000000.0(>20%) S=[illegible]

9/15 [illegible]2.00 A=130476.5(0.5%) B=130460.5(0.5%) C=000000.0(>20%) S=[illegible]

9/14 351954 x 50 x 1 = 17.6 x 10^6 counts

9/15 130460 x 100 = 13 x 10^6 counts
(Repeated rxn $\bar{c}$ phenol extracted 47.4 .6Kb E/Bg + 63.1 1.0Kb H3

Next Page

* Pooled all 47.4 labellings (2)

13 x 10^6
18.5 x 10^6
31.5 x 10^6 counts

47.4 0.690 E/BQ

[redacted]

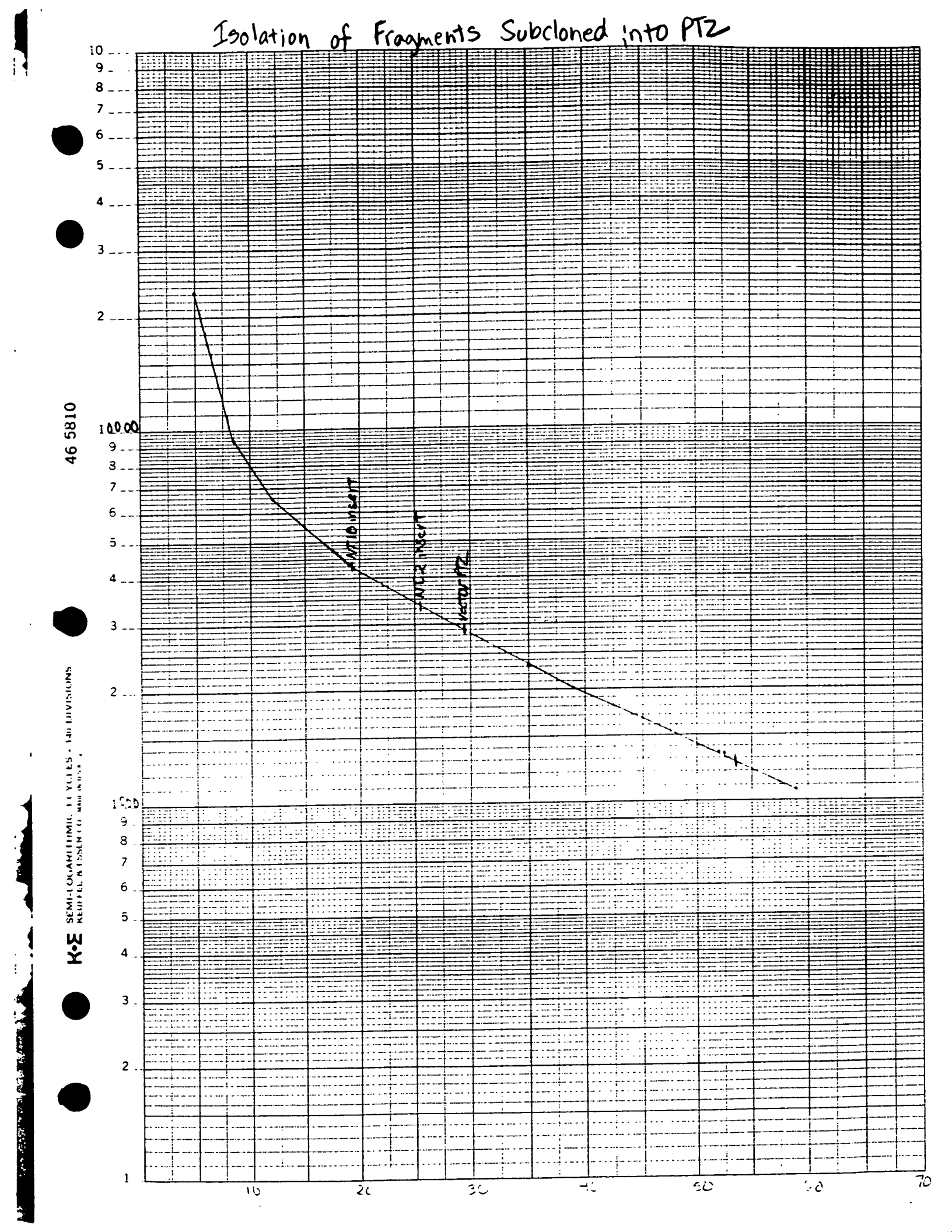
18506 x 100 x 1 = 18.5 x 10⁶ counts

63.1 1.026 H3

[redacted]

56068 x 100 x 1 = 5.6 x 10⁶ counts

Did not label well
After Lisa labelled it - no problem


Isolation of Fragments Subcloned into PTZ
46 5810
K·E
10
9
8
7
6
5
4
3
2
1000
9
8
7
6
5
4
3
2
100
9
8
7
6
5
4
3
2
1
10
20
30
40
50
60
70
Insert
PTZ insert
Vector PTZ

Mini-Prep (Gen. clone inserts, DMD inserts in PTZ)

Make soln 2 Fresh → 3.8ml → 4.0ml

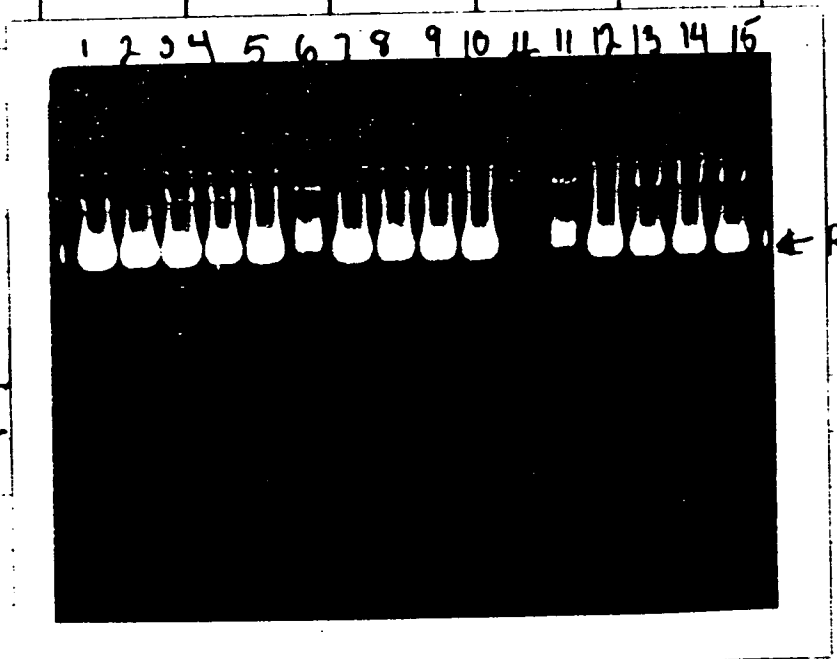
80µl 10N	60µl 10N NaOH
160µl 25% SDS	120µl 25% SDS
3760µl H_2O	2820 H_2O

Soln. 1 need 2 ml
" 3 " 3 ml

1	20	I
2	20	II
3	20	III
4	20	IV
5	20	V
6	20	VI
7	12.2	I
8	12.2	II
9	12.2	III
10	12.2	IV
11	12.2	V
12	12.2	VI
13	NT.2	I
14	NT.1b	I
15	NT.1b	VI

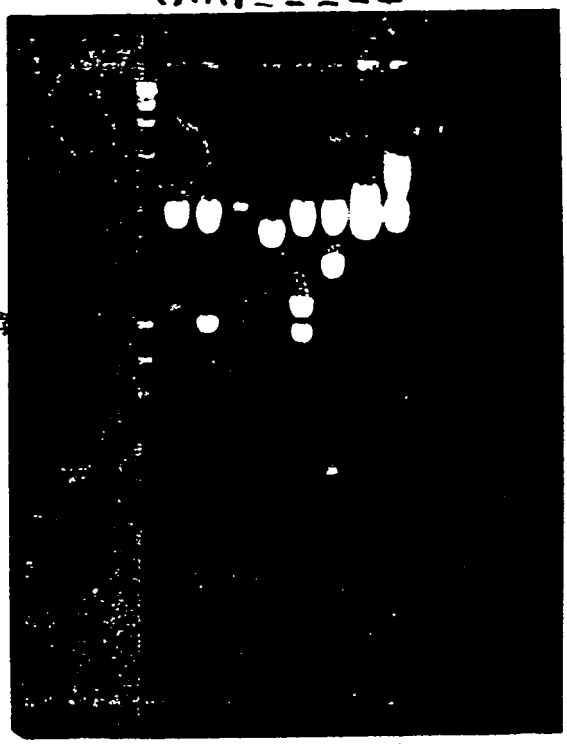
(some soln spilled (NH4Ac + DNA))

Added NH_4Ac instead of NaAc (soln 3)



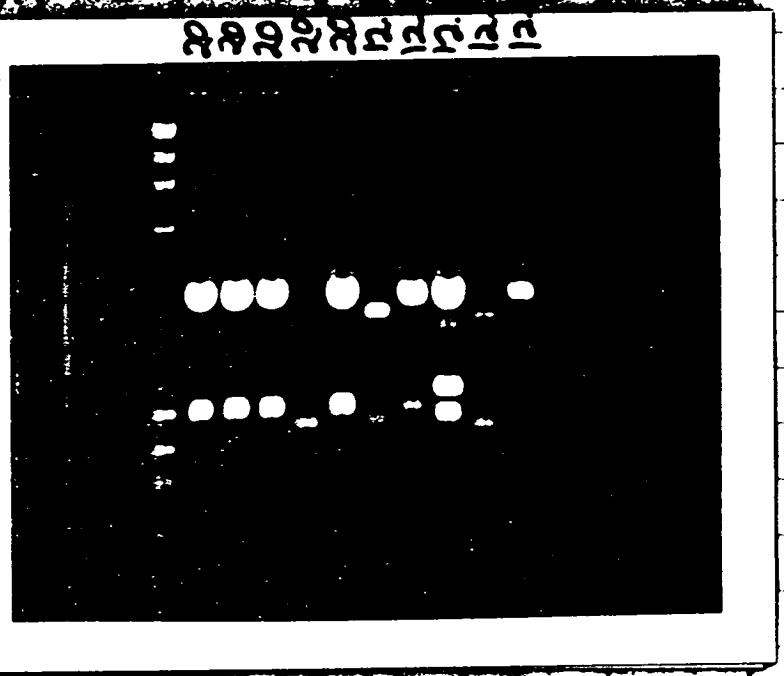
Mini-Prep Gel ε NT.1b, .2, 20, 12.2 i's

Lane		DNA	(µl)	10x Enz Buffer	(µl)	Enzyme	(µl)	Spermidine	RNAse	TE	To
1	1	STANDARD			15						
2 √ (1)	2	20	10	2	2	HT/SST	1.0 + 1.0	1	2	3	2
(2)	3	20		2	2	"		1	2		
(3)	4	20		2	2	"	"	1	2		
(4)	5	20		2	2	"	"	1	2		
3 √ (5)	6	20	10	2	2	"	"	1	2	3	20
4 √ (6)	7	20	10	2	2	"	"	1	2	3	20
(7)	8	12.2		2	2	"	"	1	2		
(8)	9	12.2		2	2	"	"	1	2		
5 √ (9)	10	12.2	10	2	2	"	"	1	2	3	20
6 √ (10)	11	12.2	10	2	2	"	"	1	2	3	20
(11)	12	12.2	1	2	2	"	"	1	2		
7 √ (12)	13	12.2	10	2	2	"	"	1	2	3	20
8 √ (13)	14	NT.2	10 lg scale prep	2	2	"	"	1	2	3	20
(14)	15	NT.1b		2	2	Hind	2	1	2		
9 √ (15)	16	NT.1b	10 lg scale prep	2	2	Hind	2	1	2	3	20
	17										
	18	Digest 2 hrs.									
	19										
	20	20	1.25Kb								
	21	12.2	2.75Kb								
	22 √	NT.2	3.35Kb								
	23 √	NT.1b	4.3Kb								
	24										
	25										
	26										
	27										
	28										
	29										
	30										
	31										



Second Mini-prep of Ligations → pIZ

	1	2	3
1	20	VII	
2	20	VIII	→ missing from freezer
3	20	IX	
4	20	X	
5	20	XI	
6	20	XII	Lg. scale prep
7	12.2	VII	
8	12.2	VIII	
9	12.2	IX	
10	12.2	XI	
11	12.2	XII	
12			
13			
14	12.2	X did not grow	
15			



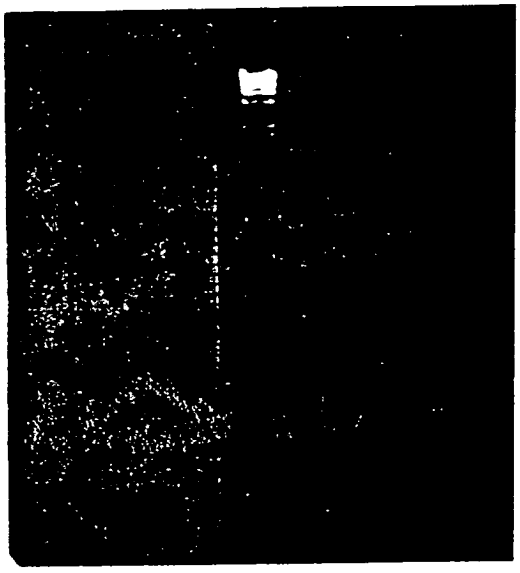
	DNA	(µl)	10x Buff	µl	Enzyme	µl	Spermidine	RNase A	TE	Tot
0	λ-Øx	15								
1	5	1-20 VII	React 2	2	H3/SST	1+1	1	2	8	20
2	5	3-20 IX	2	2	H3/SST	1+1	1	2	8	20
3	5	4-20 X	2	2	H3/SST	1+1	1	2	8	20
4	5	5-20 XI	2	2	H3/SST	1+1	1	2	8	20
5	5	6-20 XII	2	2	H3/SST	1+1	1	2	8	20
6	5	7-12.2 VII	2	2	H3/SST	1+1	1	2	8	20
7	5	8-12.2 VIII	2	2	H3/SST	1+1	1	2	8	20
8	5	9-12.2 IX	2	2	H3/SST	1+1	1	2	8	20
9	5	10-12.2 XI	2	2	H3/SST	1+1	1	2	8	20
10	5	11-12.2 XII	2	2	H3/SST	1+1	1	2	8	20
11										
29	Digest 2 hrs.									
30	1% gel									
31	Blotting									

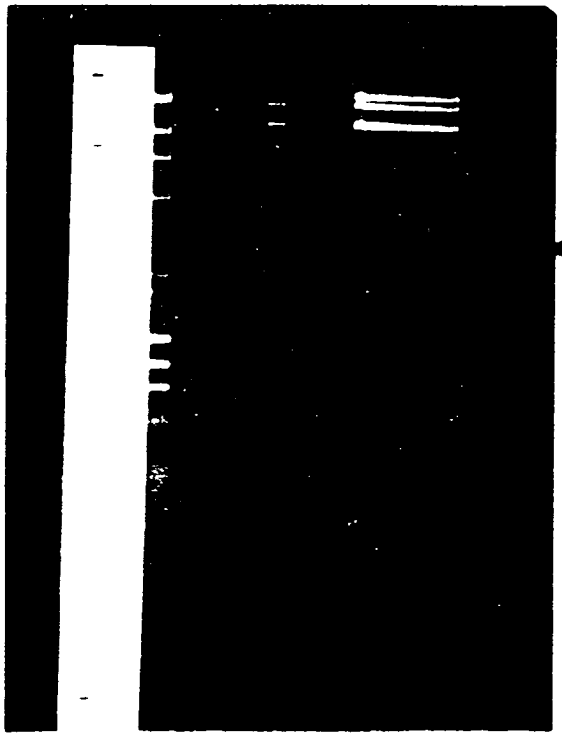
Prep Gel to Isolate 3Kb (Asp718) Frag. of 12.1

[redacted] 87

1 DNA	2 (µl)	3 10x Buff	4 (µl)	5 Enzyme	6 (µl)	7 Spermidine	8 RNAseA	9 TE	
λ-ØX	20								
12.1	10	2	5	Asp 718	5	2	3	25	50
12.1	~250	2	40	Asp 718	40	20	20	30	400

Cut out 3Kb Asp 718 fragment
Soaked in H_2O 30' - shaking ~ every 5'
Set up electrolution





← 12.1 3Kb Asp

Concentration of 12.1 3Kb Asp frag
20-25 ng/λ.

Jack Rainer

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Klenow Fill in Rxn. For 12.1 3Kb Asp (25ng/λ) [redacted]

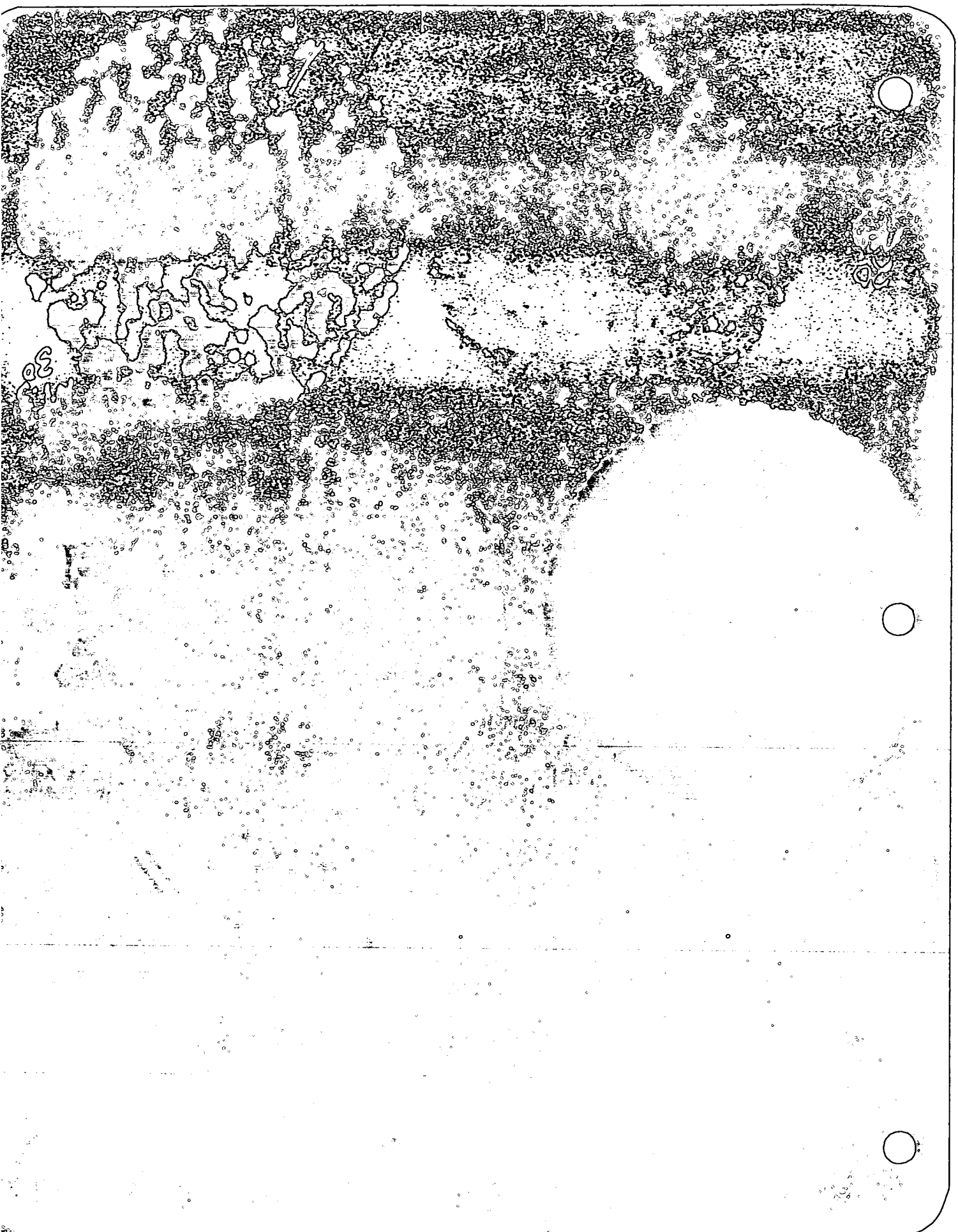
H_2O+DNA	5 μl
dNTP's	1 μl
10x Buff.	1.5 μl
Klenow	1 μl
H_2O	6.5λ
	15λ

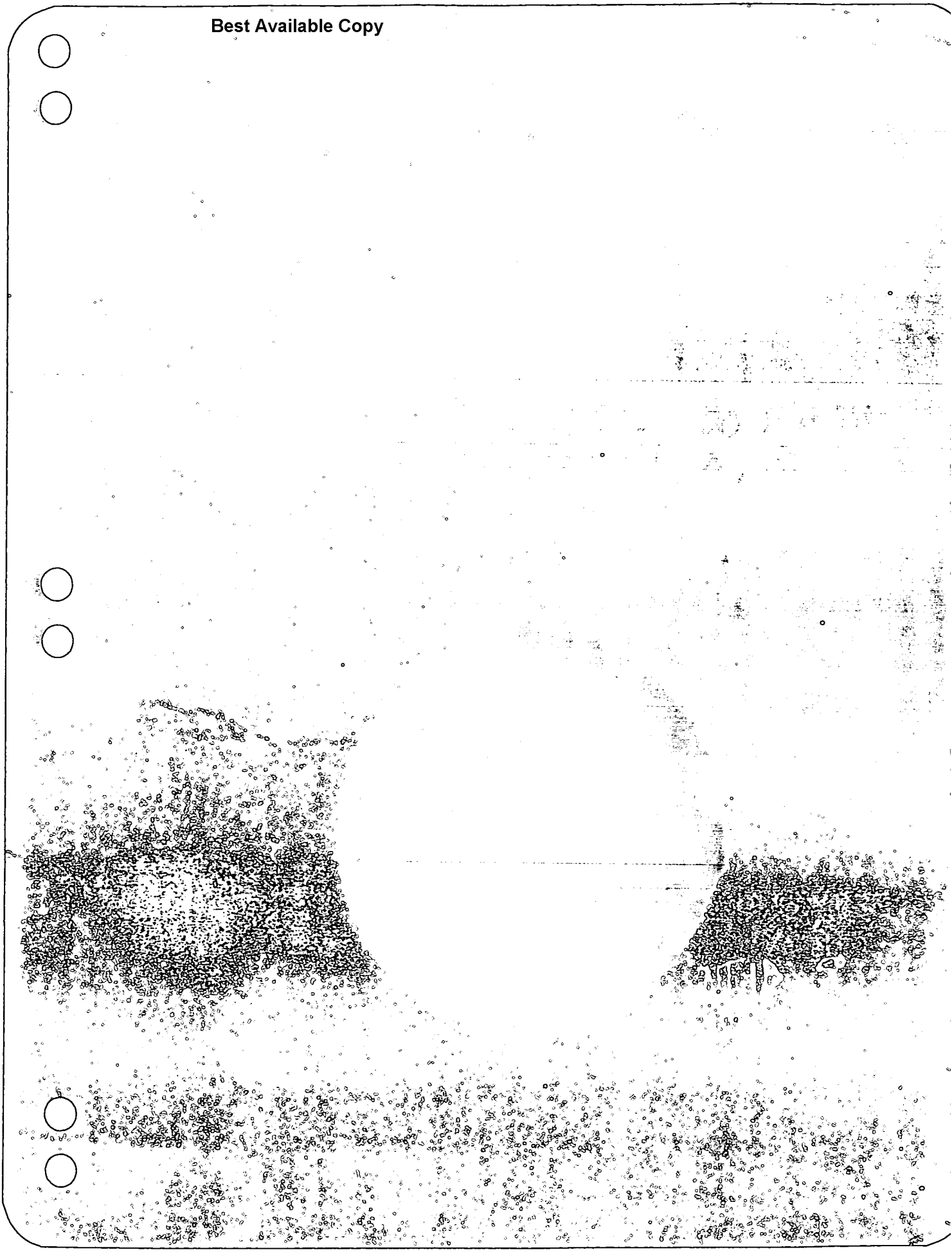
½ vol AmAc
2-2½ vol. EtOH - mix
-70°C 10 min.
Wash ε 50λ 70% EtOH
Resuspend in vol. to use for Ligation

*Could have done 10λ rxn

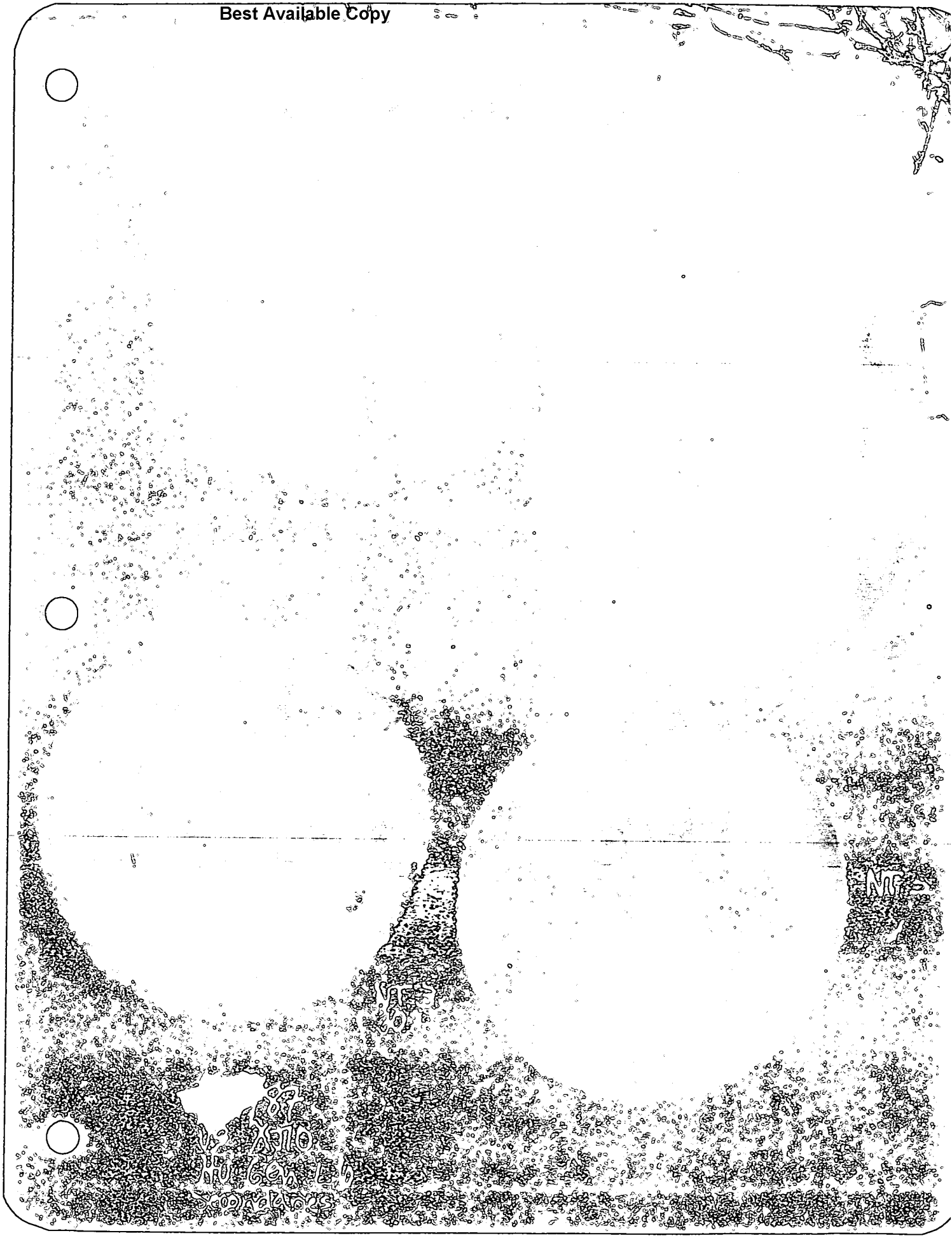
Ligation of 12.1 3Kb A into PTC18R

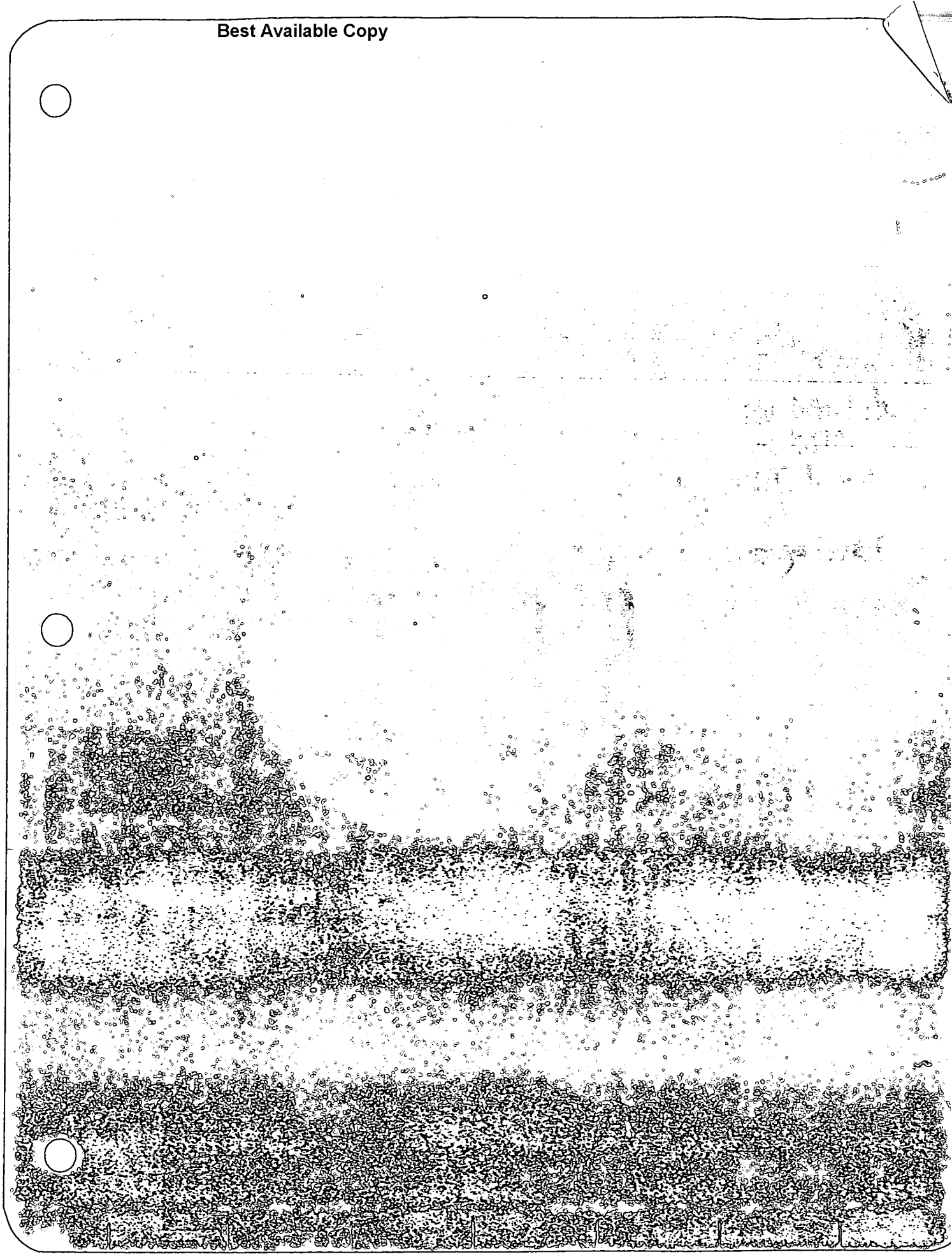
H_2O+DNA	6.5 μl
10mM ATP	1 μl
Vector	½ μl
T_4 10x Buff	1 μl
Ligase	1 μl

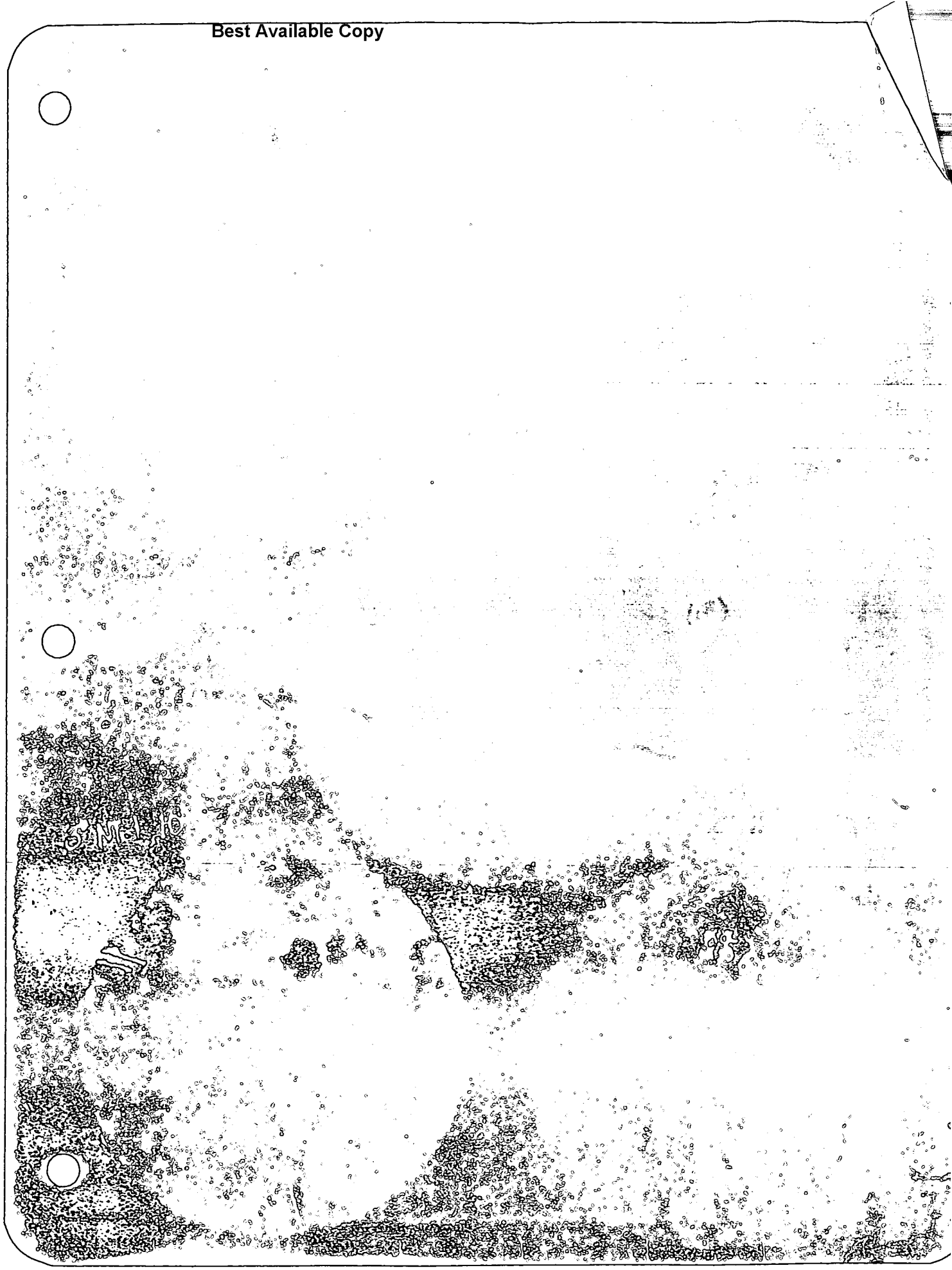


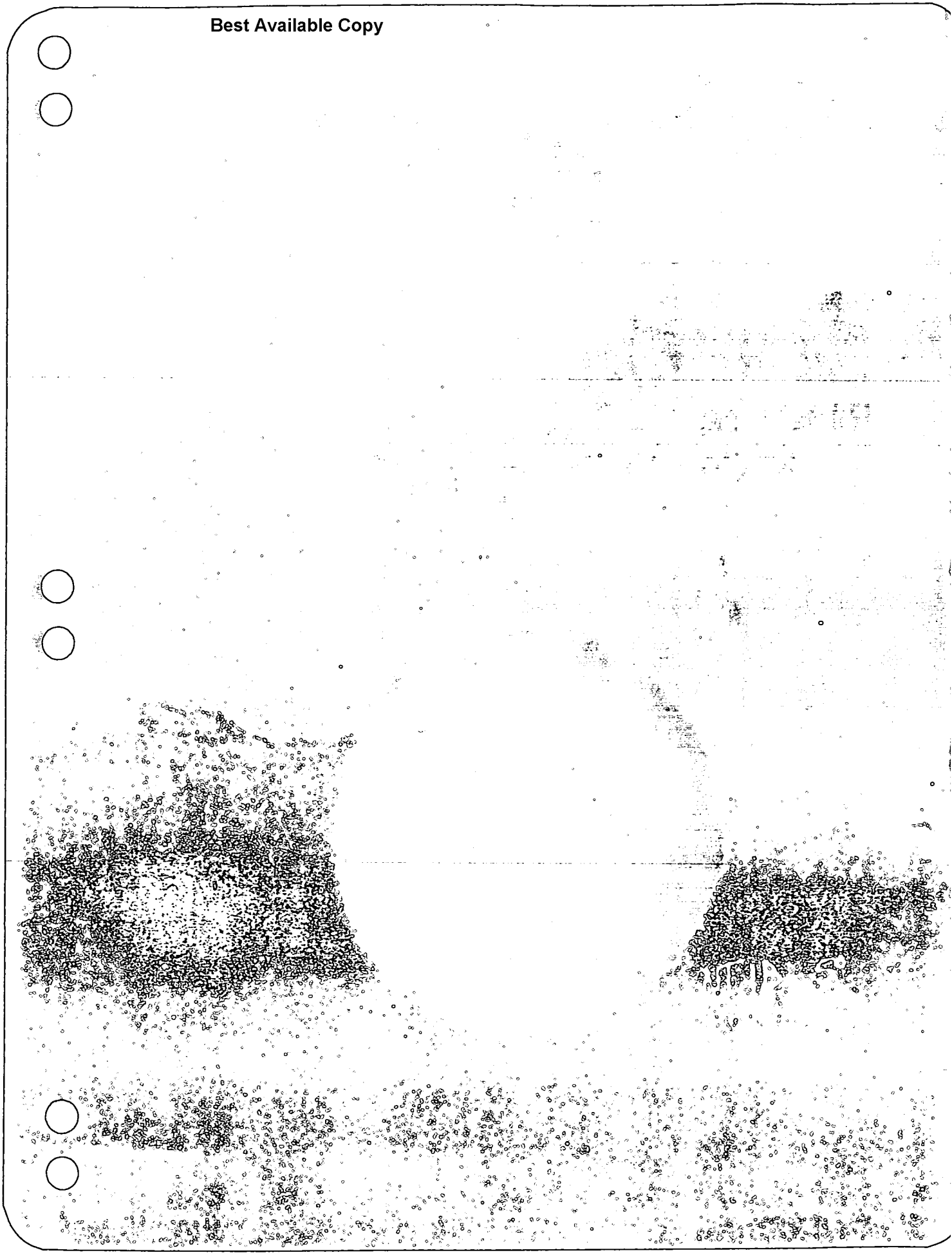


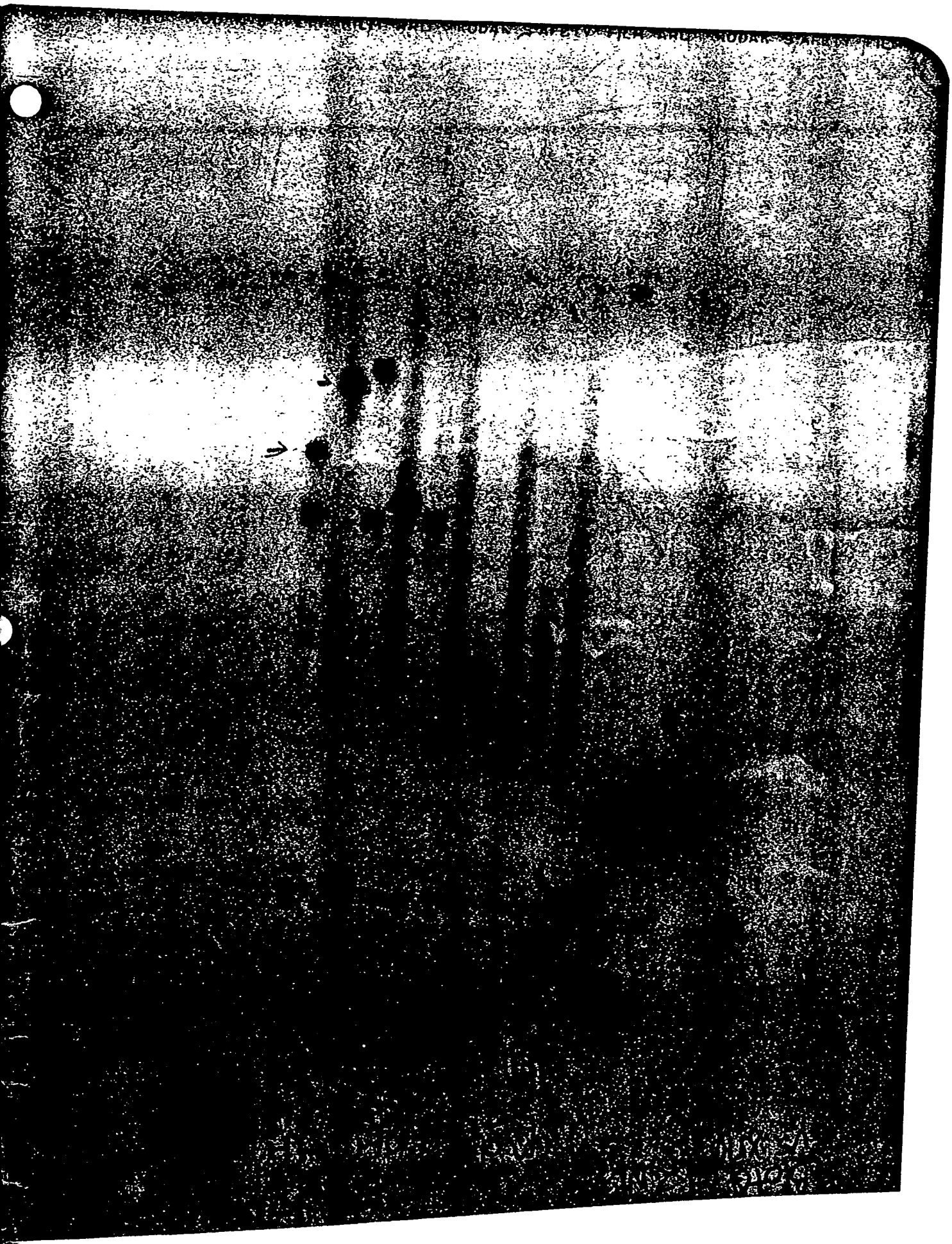

11 19.2
1/100 SA 19.2
DMD 19.2
1ST pick











Digestion of 47-4 c̄ HincII [redacted]

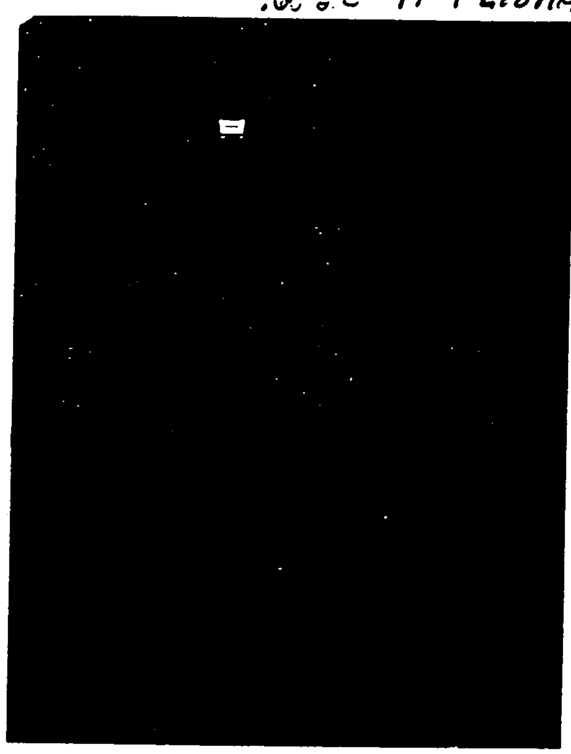
2.4 mg/ml - conc 6λ c̄ Eco, HincII, Toyobo hi salt

DNA	6λ	
10x hi	10λ	
Sp	4λ	
RNase	2λ	
EcoRI	4λ	10u/λ BRL
HincII	6λ	4u/λ NEB
H_2O	68λ	
	100λ	

5:40

.6% 47.4 EcoHincII

20λ of each
10λ to LSA



~3 kb 10/16

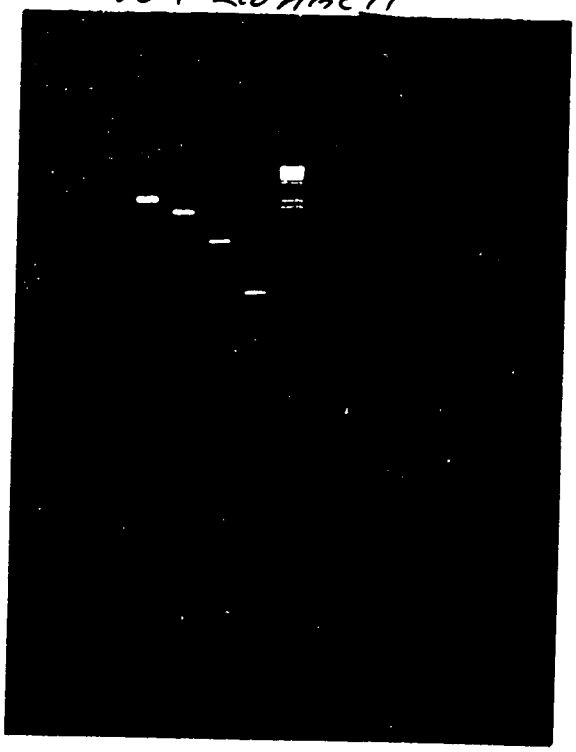
Digestion 631 c Eco-Hinc II [redacted]

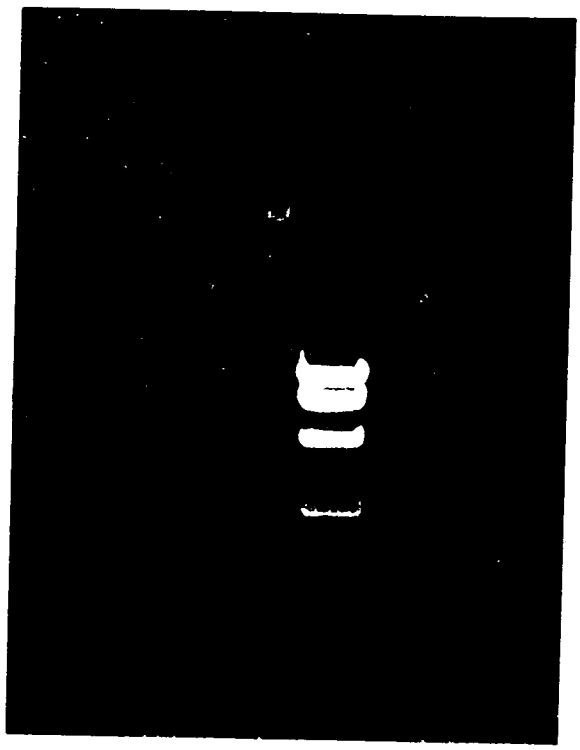
~15 mg Toyopa hi salt, 100d elute
electrolite 4 tubes (bottom 4, vector
to topoc)

~25% yield

2.5 kb	100 ng/l	20 l each
1.9 kb	40 ng/l	10 l to LSA
1.05 kb	40 ng/l	
~.6 kb	20 ng/l	

631 Eco Hinc II





Large Scale Plasmid Prep of Fragments Containing Exons Pulled out c XD-1, XJ10

[redacted]/87

For 100mls soln. 2

0.2N NaOH — 2ml 10N NaOH
1% SDS — 4ml 25% SDS
94ml H_2O

100ml soln 1

50mM	Glucose	5ml 1M
10mM	EDTA (pH 8.0)	2ml .5M
25mM	Tris-HCl (pH 8.0)	2.5ml 1M
H_2O		90.5

LYSOZYME

60mg/4ml

Problem c protein / RNA precipitating after loading into tubes for Vti 50
Spun down debris, Extracted c IsoAmyl 4x = vol.
Dialyzed o/N
Concentrated c Butanol (filled to top of tube) to 4ml.
* Added 80λ 5M NaCl (for better sep. of layers)
Extracted 3 samples c = vol. phenol (NT.1b, NT.2, 12.1)
" all samples c 2x vol. chloroform (* removes Butanol)
Precipitated c 2x vol. EtOH
Washed
Resus. in 500λ

3/200 dil	A_{260}	A_{280}	$\frac{A_{260}}{A_{280}}$	Conc.	Yield
NT.1b	1.222	.576	2.12	4.1 mg/ml	2.0 mg
NT.2	.936	.440	2.12	3.12 mg/ml	1.6 mg
NT.3	.156	.053			
20	.584	.273	2.14	1.9 mg/ml	950 µg
12.1	1.203	.580	2.07	4.01 mg/ml	2.0 mg
6/200					
NT.3	.363	.204	1.78	0.61 mg/ml	303 µg

Jozel Kanier

Hu. Gen. Clones (DMD) in PTZ19R [redacted] 87

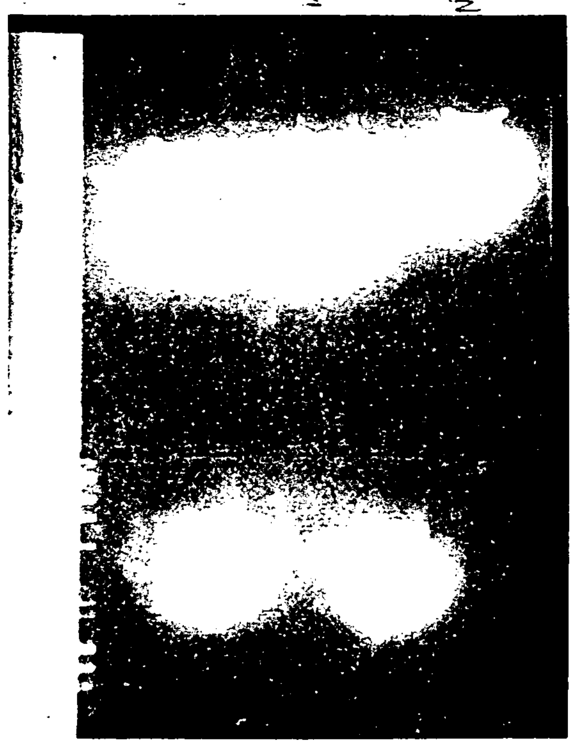
1	2	Insert	Conc.	For 10µg of insert	Conc of insert	↓	RE sites
NT.1b		4.3Kb	4.1mg/ml	(60%)	2.46mg/ml	4.1µl	HindIII
NT.2		3.35Kb	3.12mg/ml	(53.6%)	1.67mg/ml	6.0µl	H3/Sst
NT.3		3.2Kb	610µg/ml	(52.5%)	320µg/ml	31.3µl	H3/Sst
12.1		3.0Kb	4.0mg/ml	(50.8%)	2.03mg/ml	4.9µl	H3/Sst
20		1.25Kb	1.9µg/ml	(30.12%)	572µg/ml	17.5µl	H3/Sst

cut 20µg insert
+100λ digestion
+10X '2' buffer
4λ Spermidine
2λ RNaseA
5λ ea. enzy (10λ c ...

	0.2,0.3,20,12	NT.1b	+ 8.2 DNA
Buff	10x4	10	\
Enz H	5x4	10	
S	5x4	—	
Sper.	4x4	4	
RNase	2x4	2	
H_2O	19.4x4	65.8	
	37.4 in each		

	NT.1b	NT.2	NT.3	12.	
H_2O	X	50.6	—	52.8	27.6
DNA	X	12µl	62.6	9.8	35µl

5 12.1 NT3



NT.1b NT 2

* NT.2 + NT.3 were not resolved
DNA migrated strangely - curling beginning to be visible here

Hybridization of Double Blot (XJ10, XD-1, 4x4) 11/2/87

Labelling of XJ10 and XD-1 (TCA precipitated)

XD-1 T=002.00 A=137562.5(0.5%) B=137536.0(0.5%) C=000000.0(>20%)
XJ10 T=002.00 A=269291.5(0.3%) B=269260.5(0.3%) C=000000.0(>20%)

XD-1 (10% hxn+.25ET)
137526 x100 = 14x10⁶ counts
For 500,000 counts/12ml used 43.6%
240% H.S. DNA

XJ10 (100%, hxn+.25ET)
269291 x100 = 27x10⁶ counts
For 500,000 counts/12ml used 23%

(Both probes thrown in)
Hybed at 50% F
Washed 1x 2xSSC RT
1x 2xSSC 65°C 30'
1x 0.1xSSC 50°C 30'
ON Film O/N RT
+ O/N -70°C

4x4 (column) Hybed at 50% F
F1: 98882 ÷3 x975 x2 = 64x10⁶ counts → 180% for 1.2x10⁶/12ml
66,000/%
240% H.S. DNA
F2: 42,283 ÷3 x x2 =

T=002.00 A=098882.5(0.5%) B=098872.5(0.5%) C=000000.0(>20%)

T=002.00 A=042283.5(0.7%) B=042275.5(0.7%) C=000000.0(>20%)

Washed 1x RT
1x 0.1xSSC 45°C 30'

Gel Ranier

Isolation of Hu. Gen. Clone Fragments to be sequenced 14/5/87

	DNA	(μl)	10xBSA (μl)	(μl)	ENZYME	(μl)	Sp.	H_2O	total
1	ΦX	20							
2	NT.1b	10	2	2	DdeI	2	1	5	20
3	4.3Kb								
4	20	10	1	2	RsaI	2	1	5	20
5	1.25Kb								
6	12.1	8	2	2	HhaI	2	1	7	20
7	1.1Kb								
8									
9	Want	NT.1b 0.8Kb DdeI fragment							
10		20 0.6Kb RsaI " .65							
11		12.1(1.1) 0.45Kb HhaI "							
12									
13									
14									
15									
16									
17									
18									
19									
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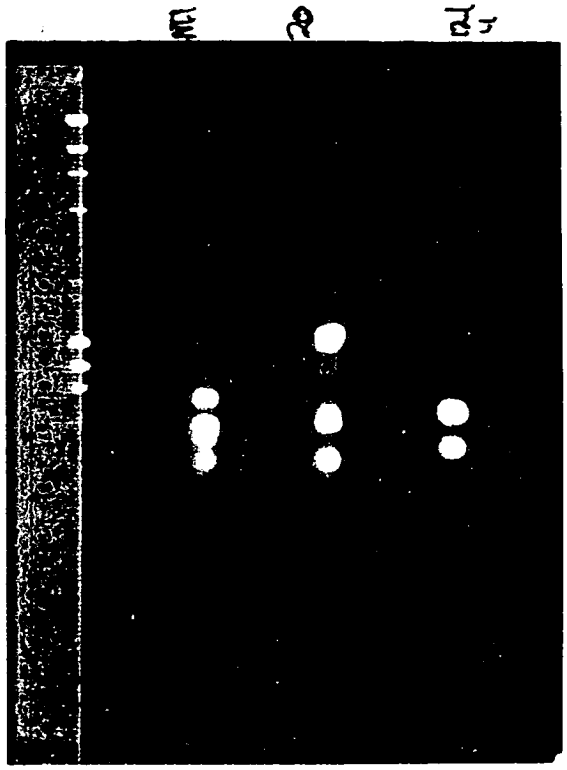


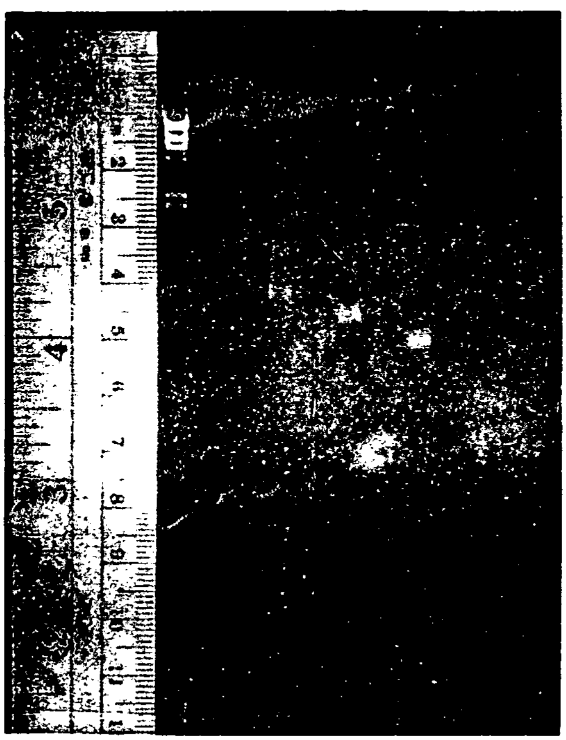
11/7/7-

1	2	Size of whole insert	RE Sites	5	Fragment	Enzyme	Amount of insert cut	Percent of insert cut out of gel	DNA expected = …% recov
NT.1b		4.3Kb	HindIII		0.85Kb	Dde I	~6μg	20%	600ng
12.1(1.1)		3.0Kb	H3/SSt	(from 1.1Kb)	0.45Kb	Hha I	3μg	41%	650ng
20		1.25Kb	H3/SSt		0.65Kb	Rsa I	~6μg	52%	1.56μg

NT1b - blunt 100ng c̄ klenow
12.1(1.1) " 50ng " T4 pol
20 " 75ng c̄ " "

10λ Rxn
1λ enzyme
1λ 20mM dNTPs
1λ 10x buffer
1hr 37°
-after- Δ65° x 10'
-add 5λ 7.5M NH_4Ac
-add 35λ eth
-ppt.

NT.1b 20 12.1



Concentrations (1/2 λ)

			Amount for Blunt
NT.1b	0.85Kb	30ng/λ	3.4λ
20	0.65Kb	20ng/λ	3.75λ
12.1(1.1)	0.45Kb	40ng/λ	1.25λ

	NT.1b	20	12.1
DNA	3.4λ	3.75λ	1.25
Enz.	1, klenow	1, T4	1 T4
dNTP's	1	1	1
10x Buff	1	1	1
H_2O	3.6	3.25	5.75

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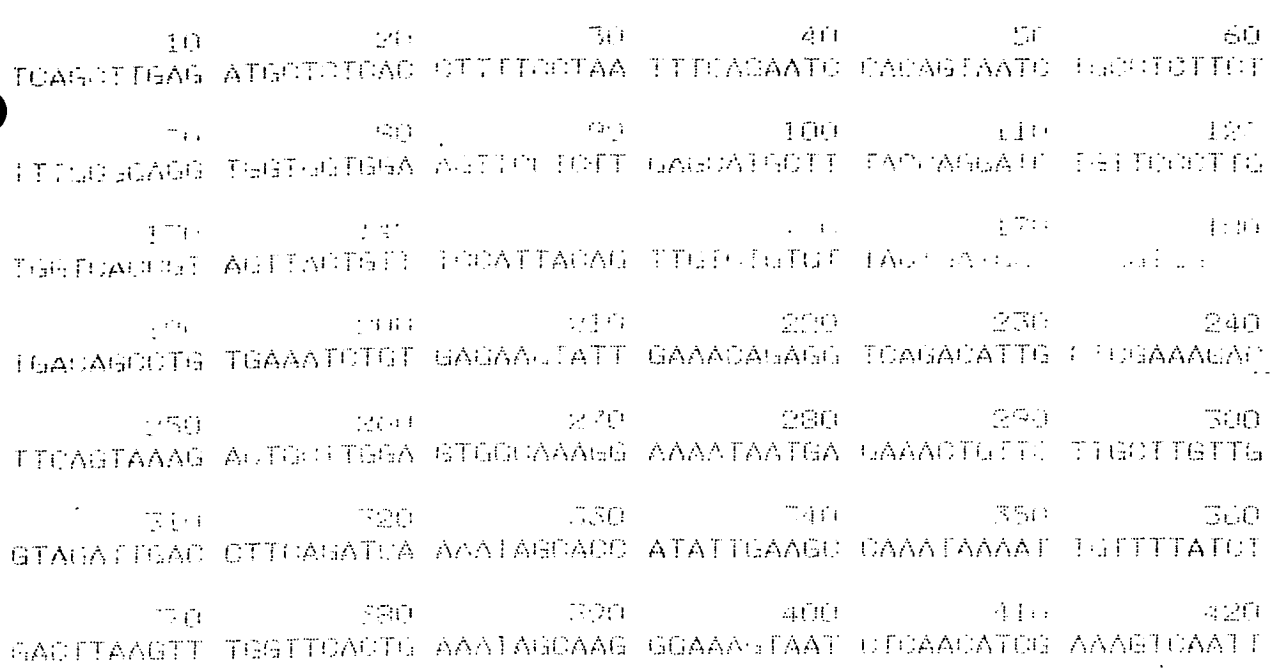


TA

Preliminary Sequencing of 2 clones from 3' 1/11/8

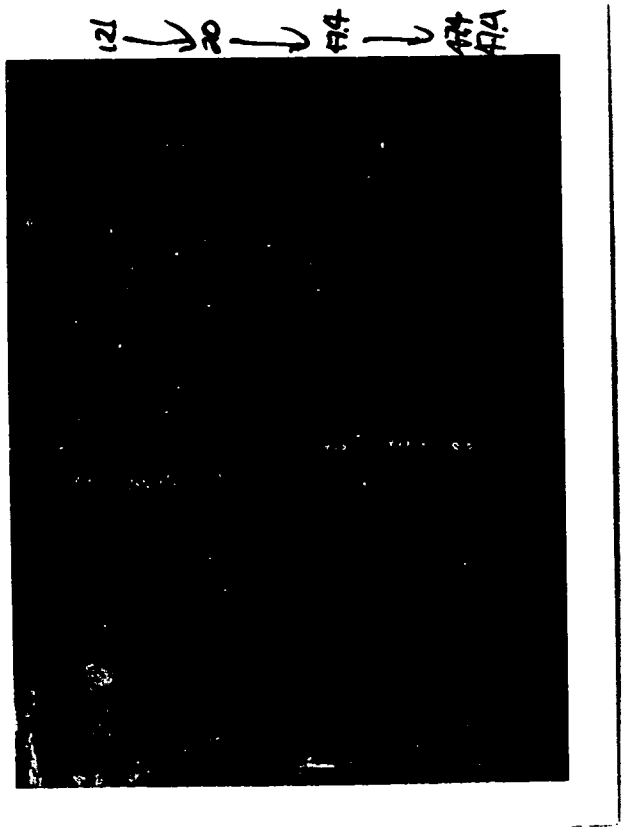
Will sequence 12.1 V a 0.45 HhaI into PTZ
NT.3 V a 0.5 DdeI into PTZ redo (T lane not clear)

1/12/8
12.1 VI b DH5α F'
47.40.2 IV a 0.2 HincII/PvuII DH5α F'
NT.3 IV a again 0.5 DdeI PTZ, DH5α F'
NT.3 I b 0.5 DdeI PTZ DH5α F'

Mini-Preps 12.1 (.45Kb), 20 (.65Kb), 47.4 (.6Kb) 11/17/7

	1	2	3	4	5	6	7	8	9
.45Kb	12.1	VI	(blue)						
2	12.1	VII		Soln.	1	1.5ml			
3	12.1	VIII			2	3 ml			
4	12.1	IX			3	2ml			
.65Kb	20	VI	(blue)						
6	20	VII							
7	20	VIII							
8	20	IX							
.6Kb	47.4	VII	(blue)						
10	47.4	VIII	"						
11	47.4	IX	"						
12	47.4	X	"						
13	47.4	XI	white						
14									
15	Gel of Mini-Preps (inserts ran off gel) over								
	DNA	(µl)	10xBuff (µl)		Enz.	(µl)	Sper.	RNase	TE
.45Kb 1	12.1	10	2	2	H3/Sst	1+1	1	2	3
2	12.1	10	2	2	"	"	"	2	"
3	12.1	10	2	2	"	"	"	"	6
4	12.1	10	2	2	"	"	"	"	"
.65Kb 5	20	10	2	2	"	"	"	"	"
6	20	10	2	2	"	"	"	"	"
7	20	10	2	2	"	"	"	"	"
8	20	5	2	2	"	"	"	"	8
.6Kb 9	47.4	5	2	2	H3	1	"	"	9
10	47.4	5	2	2	"	1	"	"	9
11	47.4	5	2	2	"	1	"	"	9
12	47.4	5	2	2	"	1	"	"	9
13	47.4	5	2	2	"	1	"	"	9
30									
31									

Mix
24 TE
16 Buff
16 RNase
8 Sper
8 H3/
8 Sst



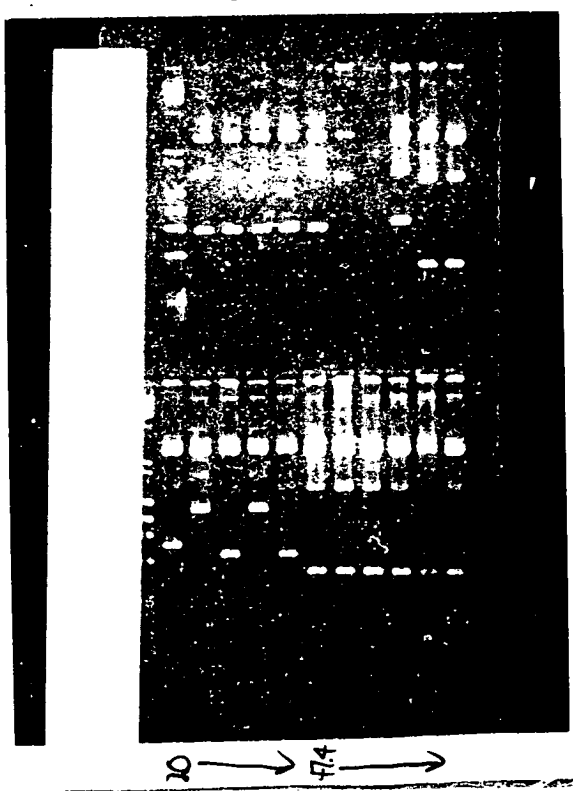
Jeff ran it too far

Joel Ranier

Mini-Prep Gel NT.1b(0.85), 12.1(0.45) 20(0.65) 47.4(0.6kb) 11/15/7

	DNA	(µl)	10xBstBS	(µl)	Enz	(µl)	Spermdine	RNAseA	TE	Total
1	λ-ΦX	20								
√(1) 2	NT.1b (0.85)	2	2	2	H3/SST	1+1	1	2	11	
√2 3	NT.1b	2	2	2	"	"	1	2	11	
√3 4	NT.1b	2	2	2	"	"	1	2	11	
√4 5	NT.1b	2	2	2	"	"	1	2	11	
√5 6	NT.1b	2	2	2	"	"	1	2	11	
6 7	12.1 (0.45)	5	"	"	"				8	
7 8	12.1	5	"	"	"				8	
8 9	12.1	5	"	"	"				8	
√9 10	12.1	5	"	"	"				8	
√10 11	12.1	5	"	"	"				8	
11 12	20 (.65)	5	"	"	"				8	
12 13	20	5	"	"	"				8	
√3 14	20	5	"	"	"				8	
14 15	20	5	"	"	"				8	
√15 16	20	5	"	"	"				8	
16 17	47.4	14	"	"	H3	1			—	
17 18	47.4	14	"	"	"	1				
14 19	47.4	14	"	"	"	1				
19 20	47.4	14	"	"	"	1				
20 21	47.4	14	"	"	"	1				
21 22	47.4	14	"	"	"	1				
23										
24	To do - pick more 47.4 0.6kbE/Bg (same blue)									
25	" 12.1(.45), 20(.65)									
26	for better chance of opp.									
27	orientations									
28										
29										
30										
31										

NT.1b → 12.1 →



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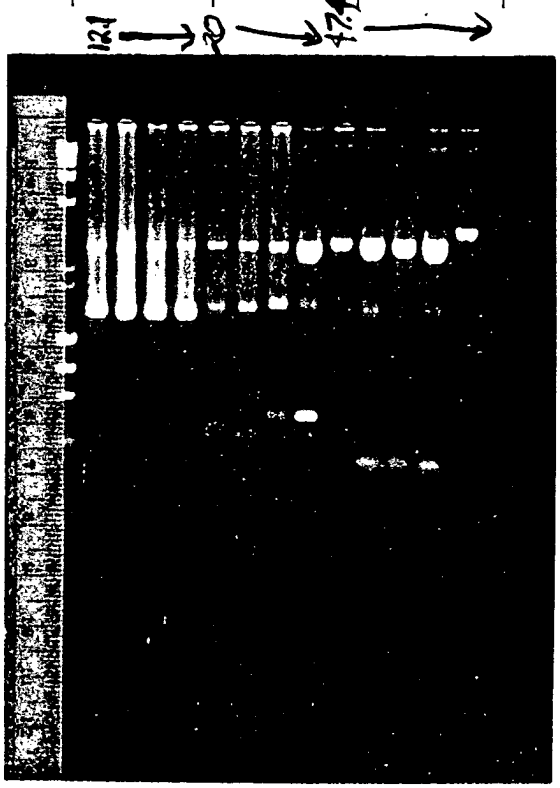
2nd Gel of Mini-Preps 12.1(.45) 20(.65) 47.4(.6) 11/29/7

(prepped 11/27)

	1	2	3	4		7	8	9
1								
2	DNA	(µl)	10X Buff	(µl)	Enz.	(µl)	Sper.	RNase A	TE
3	λ-ΦX	20						
✓1 4	12.1 (VI)	13	2	2	H3/SstI	1+1	1	2	—
✓2 5	12.1 (VII)	13							
✓3 6	12.1 (VIII)	13							
✓4 7	12.1 (IX)	13							
✓5 8	20 (VI)	13							
✓6 9	20 (VII)	13							
7 10	20 (VIII)	13							
8 11	20 (IX)	10			↓	↓			3
9 12	47.4 (VII)	10			H3	2			
10 13	47.4 (VIII)	10							
11 14	47.4 (IX)	10							
12 15	47.4 (X)	10							
13 16	47.4 (XI)	10	↓	↓	↓	↓	↓	↓	↓
17									
18	12.1	0.45kb	0.5?						
19	20	0.65kb							
20	47.4	0.6kb	0.55						
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



Joel Ranier

C-Tests on Clones 12.1(0.45), 20(0.66), + NT.1b(0.85) 12/11/7

DH5αF' TG-1

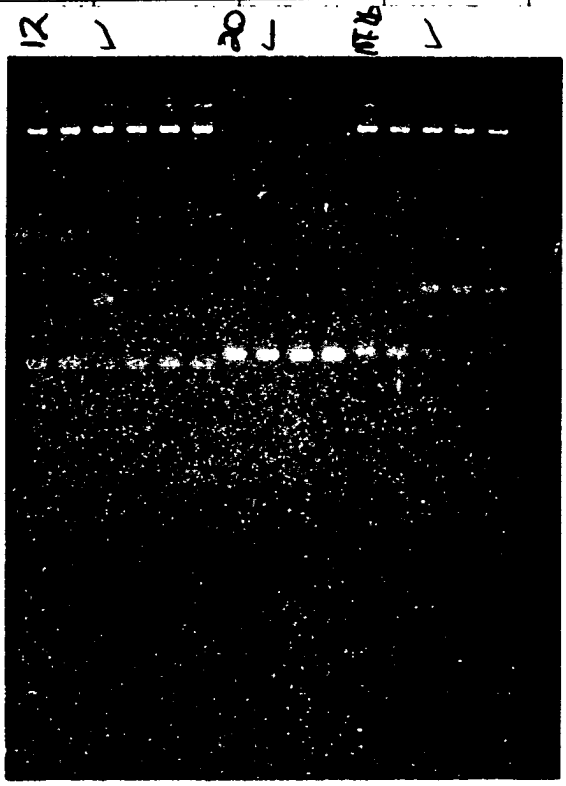
Iml of 3ml culture grown up to 0.5-0.8 OD
Infected c Helper Phage and grown o/N
Supernatant from tubes not to be used for purification used for C tests

	1	2	3	4	5	6	7	8	9
	✓A 12 V	B 12 III	✓C 12 VI	D 12 IX	E 12 IV	F 12 VIII			
20µl	10	10	10	10	10				
A→	10	10	10	10	10				
	✓G 20 III	H 20 V	I 20 VI	J1 20 III					
20	10	10	10						
G→	10	10	10						
	✓J2 NT.1b I	K NT.1b V	✓L NT.1b II	M NT.1b IV	N NT.1b III				
20	10	10	10	10					
J2→	10	10	10	10					

65°C 1hr c 1x 2% SDS
25% Dye

Vortex, spin 30 sec.

Run .9% gel



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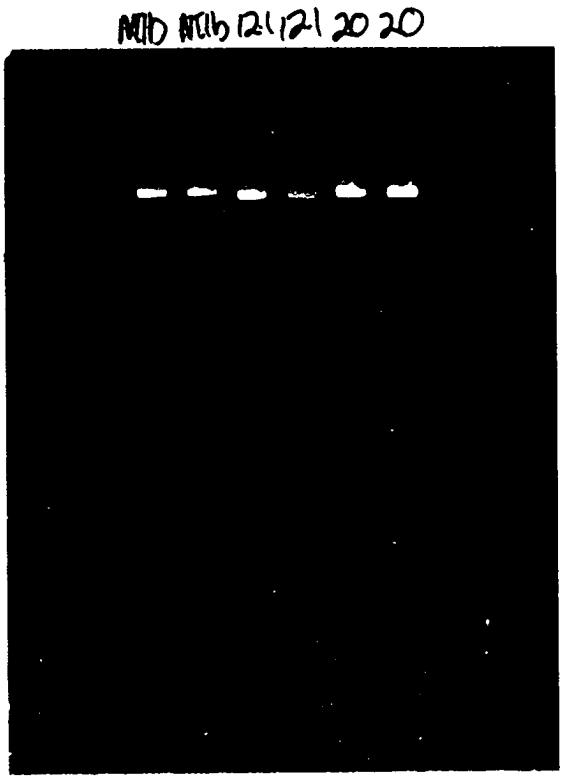
Purification of DNA for Sequencing 12/18/7

1↗

NT.1b I & II
(opp. orientations)

12.1 V+VII
(opp. orientations)

20 V+VII
(not opp.)



Joel Ravin

Screening of 4X Hu. Gen. Lib. (λ-zap) 11/2/87

Plated out ~ 46,000 plaques / 11 plates

Lifted onto nylon filters (2x)
Hybridized at 40% Formamide 12 hrs.
Washed 2x RT 5' 2xSSC
2x 60°C 20' 2xSSC
1x 45°C 15' 0.1xSSC

(11 - lost T. Agar
Condensaton on another plate)

Labelling A7.4 0.6 KB E/Bg fragment

101589 x 975 ÷ 3 x 2 = 66 x 10^6 counts
68000 / λ
650 λ for 44 x 10^4 counts / 44 ml
880 λ HS. DNA

F_1

A=101589.0(0.5%) B=101575.0(0.5%) C=000000.0(>20%)

F_2

A=031934.0(1.0%) B=031929.5(1.0%) C=000000.0(>20%)

18 Positives - Picked 10
ON, TW, TH, FR, FV, Sx, SN, NN.1, NN.2, TN

Screening of Secondaries, Tertiaries 4x 11/10/87

Lib 3. vs 47.4 0.6KB E/Bg

Titer of 1° pk → 10×10^6/ml
10×10^3/λ 10,000/λ

Secondary screen pos. for all except TW, FR
Will ~~re~~screen FR (more dense 1/100, 10λ) ~ 3°

Titer of 2° pk → 5×10^5/ml
500/λ

474 – 68000 counts/λ
want 5×10^6/10 ml

80λ Probe
200λ H.S. DNA

Approximate titer of 90 clones from 4x Lib. 11/17/7
(to make amplified phage stock)

For 1500-2000 Φ
replated
7λ (gave 9Φ), 100λ

ON
100 x 100 x 100 = 1.0 x 10⁷/ml
1000/λ

IW
X

300 IH
32 x 100 x 100 = 3.2 x 10⁷/ml
320/λ 6λ

200 FR
2500 x 100 x 100 = 2.5 x 10⁷/ml
250/λ 8λ

200 FV
56 x 100 x 100 = 5.6 x 10⁷/ml
560/λ 3λ

200 SX
68 x 100 x 100 = 6.8 x 10⁵, 680/λ 3λ

100 SW
63 x 100 x 100 = 6.3 x 10⁵/ml
630/λ 3λ

91 ~~100~~ NN.1
200 x 100 x 100 20 x 10⁶/ml
2000/λ 100λ of 1/100, Replate 15

1500 NN2
55 x 100 x 100 = 5.5 x 10⁵, 550/λ 3λ

IN
1506 15 x 100 x 100 = 1.5 x 10⁵, 150/λ 10λ

Titers of Amp. Phage Stocks 12/3/7

ON

1/100, 1λ — 200 x 91.00 x 100 = 1.82×10^{6}/ml

100,000 → ~~27λ~~ 60

IH

1/10³, 18λ — 20 x 55.56 x 10^{3} = 1.10×10^{6}/ml

For 100,000 → ~~46.0λ~~ 100λ

FR

1/10³, 53λ — 36 x 18.87 x 10^{3} = 6.8×10^{5}/ml

For 100,000 → ~~74λ~~ 150

FV

1/10³, 20λ — 16 x 50 x 10^{3} = 8×10^{5}/ml

For 100,000 → ~~63λ~~ 150

SX

1/10³, 20λ — 9 x 50 x 10^{3} = 4.5×10^{5}/ml

100,000 → 200λ

SN

1/10³, 75λ — 6 x 13.33 x 10^{3} = 8×10^{4}/ml

To reamp 15000 → take 19λ

Titers of Amp. Phage Stocks (con't) 12/3/7

NN1

$1/10^4$, 14λ

$12 \times 55.56 \times 10^4 = 6.67 \times 10^6$/ml

For 100,000 → ~~1/10, 80λ~~ 20λ

NN2

$1/10^5$, 40λ

$8 \times 25 \times 10^5 = 2 \times 10^7$/ml

For 100,000 → ~~1/10, 30λ~~ 10λ

TN

$1/10^5$, 100λ

$10 \times 10 \times 10^5 = 1 \times 10^7$/ml

For 100,000 → ~~5λ → 1/10, 50λ~~ 15λ

FI.1

10^3, 50

$300 \times 20 \times 10^3 = 6 \times 10^6$/ml

For 100,000 → ~~1/10, 83λ~~ 20λ

FI.2

10^3, 15λ

$475 \times 66.67 \times 10^3 = 3.17 \times 10^7$/ml

For 100,000 → ~~1/10, 16λ~~ 5λ

Phage Lysates (474 0.5kb clones + 63.1 1.0kb (2)) 12/4/7

Lowest plate – 40,000 ø
Highest plate – just confluent

Should have aimed for 300,000 instead of 100,000
Don't do amplification ☐ plate ☐ less than 500 ø

Eluting ☐ 10 mls SM for lysate

→ Estimated vols. of phage lysate

ON → 12 mls	stock 1mg/ml	→ 12 µl	
TH → 11.0 mls		→ 11.0 µl	
FR → 11.5 mls		→ 11.5 µl	
FV → ~~10.0 mls~~ 9.0		→ ~~100 µl~~ 9.0 µl	
SX → ~~11.5 mls~~ 10.5		→ 10.5 µl	
NN.1 → ~~11.5 mls~~ 11.25		→ 11.5 µl	
NN.2 → 11.~~25~~ 00 mls		→ 11.00 µl	
FT.1 → 10 mls		→ 10 µl	
FT.2 → 10.75 mls	10.5	→ 10.75 µl	10.5
TN → 11.75 mls	11.5	→ 11.75 µl	11.5

FT.1	10
FV	90
FT.2	10.5
NN.2	11.00
TH	11.0
FR	11.5
SX	10.5
NN.1	11.5
TN	11.5
ON	12

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O.D.'s 5l → 200 of Phage lysate DNA 12/5/17

	260	260/280	conc	yield
ON	2.504	1.879	5.0 mg/ml	1.0 mg
TH	2.555	1.809	5.1 mg/ml	1.0 mg
FR	2.585	1.677	5.2 mg/ml	1.1 mg
FV	2.585	1.71	5.2 mg/ml	1.1 mg
SX	2.545	1.90	5.1 mg/ml	1.0 mg
NN.1	1.796	2.276	3.6 mg/ml	0.72 mg
NN.2	2.394	1.915	4.8 mg/ml	0.96 mg
TN	1.929	2.27	3.9 mg/ml	0.78 mg
FT.1	2.073	2.22	4.1 mg/ml	0.82 mg
FT.2	2.307	2.00	4.6 mg/ml	0.92 mg

Ligating → pUC18

47.4 Genomic Clone Fragments for ~~Blunt-ending~~ 1/11/18

	1	2	Insert 3	4	Enzyme 5	6	Concentration	Yield 8
1	ON		4.36Kb		Eco		~25ng/λ	250ng
2								
3	FV		3.0Kb		Eco		~10ng/λ	100ng
4								
5	NN.1		3.8Kb		Eco		~7.5ng/λ	75ng
6								
7	TN		~17Kb		Eco		~50ng/λ	500ng
8								
9								
10								
11	Cut	100λ	of each				pUC18 Eco (~ 200ng/λ)	
12	ideally	want to use	150ng				bc ~7 < 7	
13	here use	5λ					dilute 1/10 → 2.5λ for 50ng	
14							1/5 → 1.25	
15								
16	DNA	5λ						
17	10mMATP	1λ						
18	pUC18	~~1.25~~	1.25λ					
19	10xLig	1λ						
20	T4 Lig	1λ						
21	H_2O	.75λ						
22		10λ						
23								
24								
25								
26								
27								
28								
29								
30								
31								

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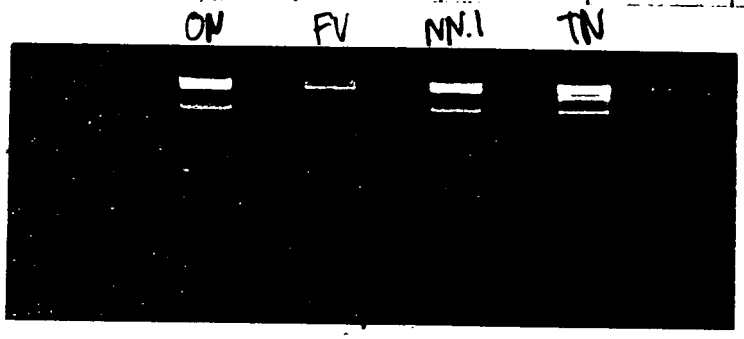
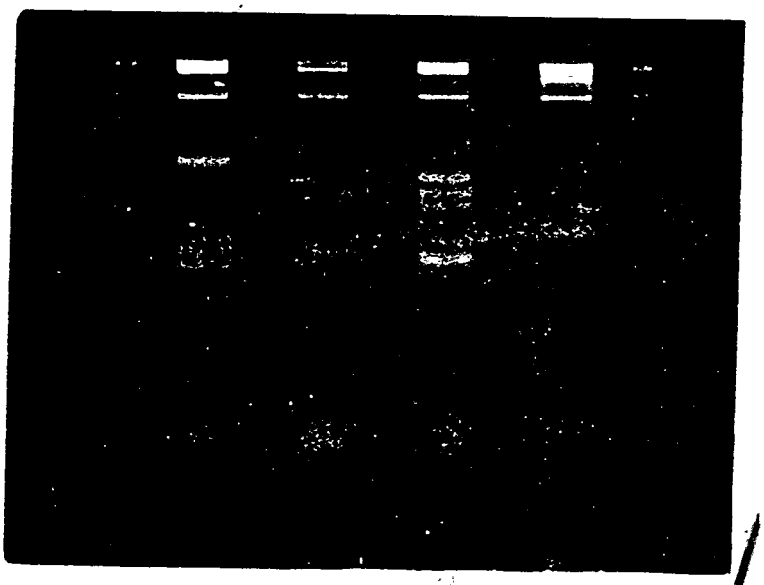
Prep Gel of 474 clones ON, FV, NN.1, TN 12/28/7

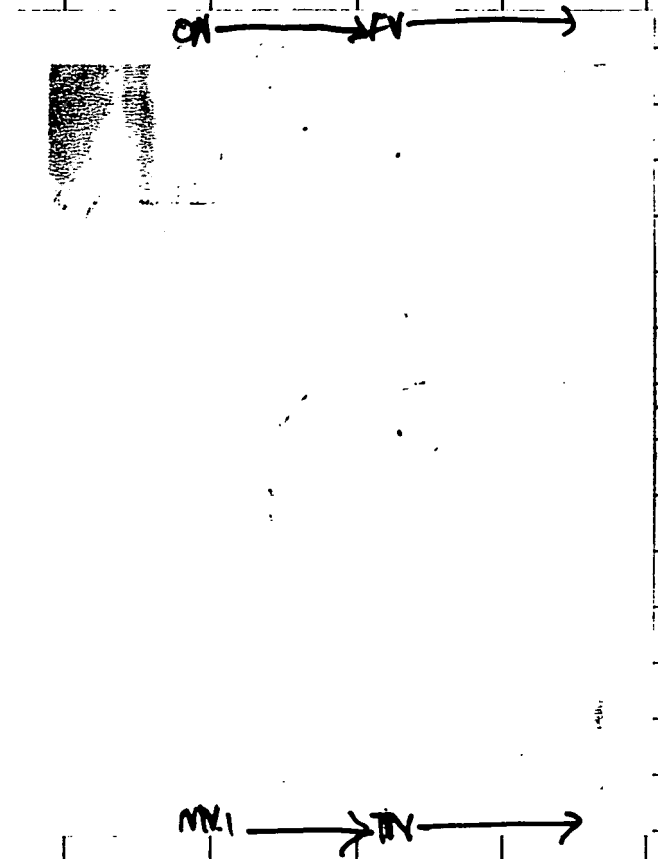
	DNA	(µl)	10XBUFF	ENZYME	(µl)	Ster.	RNase A	TE	TOT
1	ØX-λ	15							
2	ON	100	3,20	EcoRI	20	8	5	47	200
3	4.36Kb								
4	FV	100	3	"	"	"	"		200
5	3.0Kb								
6	NN.1	100	3	"	"	"	"		200
7	3.8Kb								
8	TN	100	3	"	"	"	"		200
9	~17Kb								
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									





Mini-Prep of 47.4 clones ON, FV, NN, TN 1/19/8

	1	2	3 in pUC18	6	7	8	9
1							
2	ON	I					
3	(4.36)	II		For 5ml soln 2			
4		III					
5		IV ✓		10N NaOH 100µl			
6		V		25% SDS 200µl			
7		VI		H_2O 4700			
8	FV	I ✓		5ml			
9	(3.0)	II					
10		III					
11		IV					
12		V					
13		VI					
14	NN, 1	I					Mix (23)
15	(3.8)	II					10x '3' 46
16		III ✓					Sper. 46
17		IV					RNaseH 23
18		V					TE 184
19		VI					299
20	TN	I ✓					Add 37
21	(~17)	II X					DNA 5λ
22		III					Eco 2λ
23		IV					
24		V					
25		VI					
26							
27							
28	Fragments ligated into pUC18 Eco-cip						
29	Cut E Eco						
30	2.5 hr. digest						
31							



Large Scale Prep of 474 0.6kb E/Bg Subclones 1/25/10

	1	2	3	4	5	6	7	8	9
1									
2	Lg. prep of		ON (IV) 4.3k						
3			FV (I) 3.0						
4			NN.1 (III.) 3.8						
5			TN (I) ~17						
6									
7	ON -	33.5	5.15g NH_4Ac						
8	FV -	33.5	"				4.85 g NH_4Ac		
9	NN.1	31.5ml	after remove				↓		
10	TN	35.5ml	sup before NH_4Ac				5.47g NH_4Ac		
11									
12	ON FV -		16.75 ml	7.5M NH_4Ac					
13	NN.1		15.75 ml	"					
14	TN		17.75 ml						
15									
16	ON	49							
17	FV	48							
18	NN.1	46							
19	TN	57							
20									
21	After	dialysis		For .1M NaCl					
22	ON.	5.75		115 µl of 5M				3ml NH_4Ac,	RBB
23	FV	5.75		125µl				"	18
24	NN.1	6.25		125µl				3.25 ml	19.5
25	TN	3.75		75µl				1.9 ml	11.5
26									
27									
28									
29									
30									
31									

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Concentration of Lg. Preps ON, FV, NN.1, TN 1/31/8

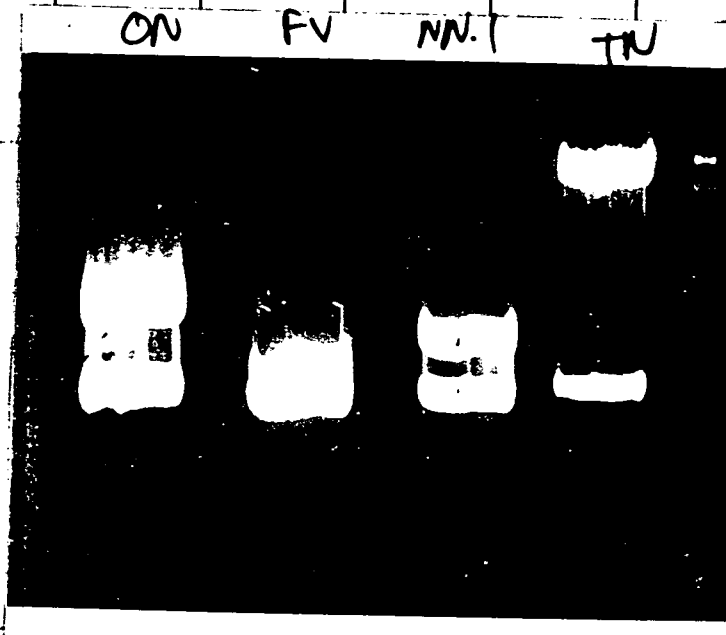
	1	2	(47.4 clones)	5	6	7	8	9
1			A260	A260/A280		Yield		Conc
2								
3	ON		2.492	1.674		1.25mg		2.5mg/ml
4	4.36							
5	FV		2.18	1.91		1.10mg		2.2mg/ml
6	3.0							
7	NN.1		.440	2.65		220µg		440µg/ml
8	3.8							
9	TN		1.80	2.01		900µg		3.6mg/ml
10	~17							
11	ON, FV, NN.1		10λ → 200					
12	TN		5λ → 200					
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

gel Rainer

Excision of Inserts from 47.4 clones

2/1/88

	1	2	3 Insert	Conc	Conc of Insert	% Insert	For 10µg Insert	RE sites	9
1									
2	ON		4.36	2.5mg/ml	1.5mg/ml	(60%)	6.7µl	EcoRI	
3	(4.36)								
4									
5	FV		3.0	2.2mg/ml	1.1mg/ml	(50.8%)	9.1µl	EcoRI	
6	(3.0)								
7									
8	NN.1		3.8	440µg/ml	250µg/ml	(56.7%)	40µl	EcoRI	
9	(3.8)								
10									
11	TN		~17	3.6mg/ml	3mg/ml	(85.4%)	3.3µl	EcoRI	
12	(~17)								
13									
14									
15	cloned into EcoRI site pUC8								
16									
17	Mix								
18									
19	10x'3'	10	x4	40					
20	Sper	4	x4	16					
21	RNaseA	2	x4	8					
22	H_2O	24	x4	96					
23		ON	FV	NN.1	TN				
24	DNA	14	18	50	7				
25	H_2O	36	32	—	43				
26	Mix	40	40	40	40				
27	Enz	10	10	10	10				
28	(Eco)								
29	Cut Appox. 20µg DNA								
30									
31									



Concentration of NN.1, TN, FV 2/12/8

	1	2	3	Conc	5	Yield	7	8	9
1	Loaded 0.5λ								
2	FV	(3.0)		~~50~~ 100ng/λ	(40λ)	~~400ng~~	2mg		
3									
4	NN.1	(3.8)		~~50~~ 100ng/λ	(25λ)	~~250ng~~	1.25mg		
5									
6	TN	(~1.7KB)		100ng/λ	(25λ)	25μg			
7				(after gel 3/2		looks like 50ng/λ)			
8						~1μg remaining			
9									
10									
11									
12									
13									

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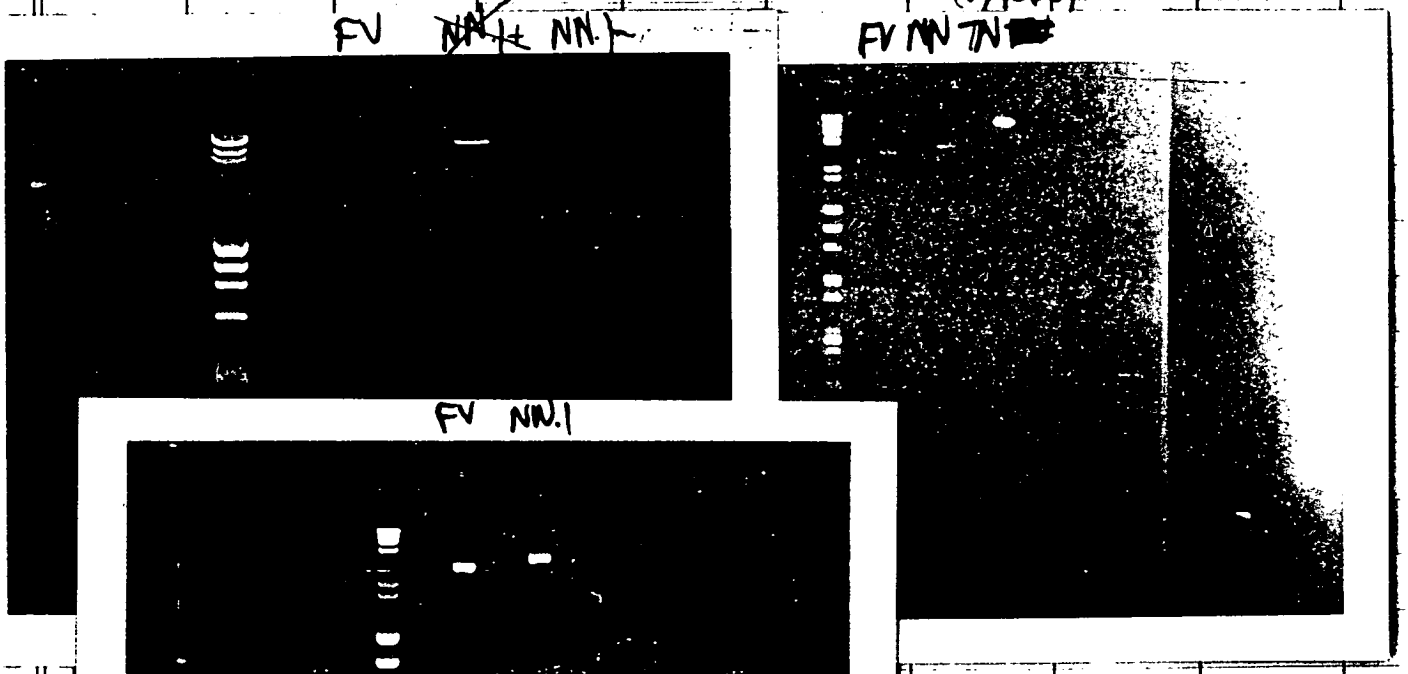

26	
27	← Re-isolation of FV, NN.1
28	.5λ of 50λ
29	200ng/λ
30	
31	

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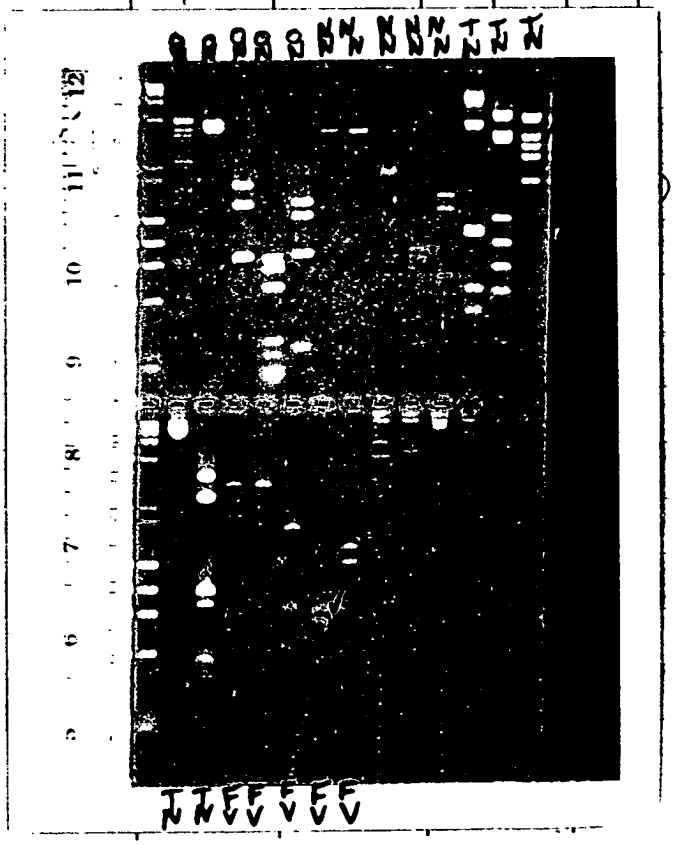
Gel of 47.4 subcloned fragments c 4 base cutters 2/28/8

	1	2 + TN	3 6 base cutters	4	5	6	7	8	9	
1										
2	DNA	(µl)	10xBuff	(µl)	Enzyme	(µl)	Sper	RNase	TE	TOT
3										
1	ON	1.5	1	2	RsaI	1	1	1	13.5	20
2	ON	1.5	2	2	HhaI	1	1	1	↓	↓
3	ON	1.5	2	2	TaqI	1	1	1	↓	↓
4	ON	1.5	2	2	DdeI	1	1	1	↓	↓
5	ON.D	1.5	3	2	BstNI	1	1	1	↓	↓
6	MN.1	5	1	2	RsaI	1	1	1	10	20
7	NN.1	5	2	2	HhaI	1	1	1	↓	↓
8	NN.1	5	2	2	TaqI	1	1	1	↓	↓
9	NN.1	5	2	2	DdeI	1	1	1	↓	↓
10	NN.1	5	3	2	BstNI	1	1	1	↓	↓
11	TN	3	2	2	HindIII	1	1	1	12	20
12	TN	3	2	2	PstI	1	1	1		
13	TN	3	3	2	BglII	1	1	1		
14	TN	3	3	2	BamHI	1	1	1		
15	TN	3	2	2	H3+PstI	1	1	1		
19		250 ng								
16	FV	8	1	2	RsaI					
17	FV	8	2	2	HhaI					
18	FV	8	2	2	TaqI					
19	FV	8	2	2	DdeI					
20	FV	8	3	2	BstNI					
25										
26										
27										
28										
29										
30										
31										



474 clones
vs. CDNA
(0.4kb EBV)
1 hr exp
-70°C
0.1 x SSC, 65°C
3/25/8

TN TN FV FV FV FV FV

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2/28/18

	1	(2 µL)	10X	(4 µL)	5	6	Sp	RNase	9 TE	
1	FV	40	2	10	DdeI	10	48	3	27	100
2										
3	12.1	100	2	20	HindIII	20	7	5	48	20
4	Phage lysate									
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
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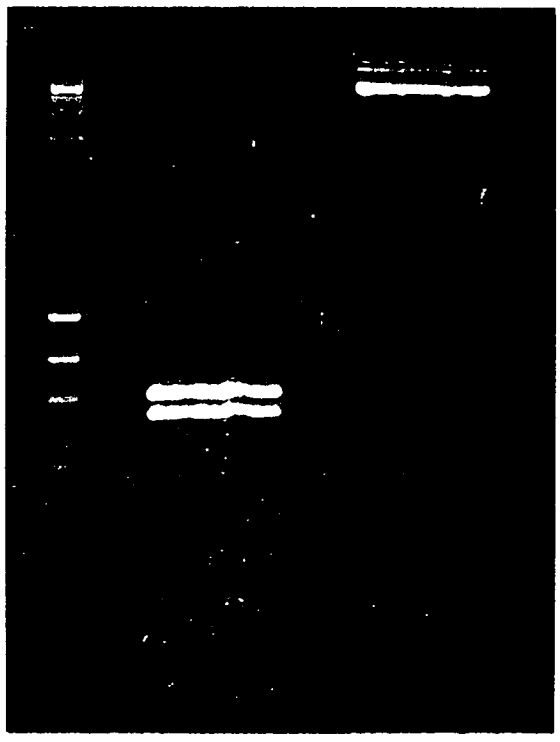
31										

0.9 DdeI →



Jozel Ramirez

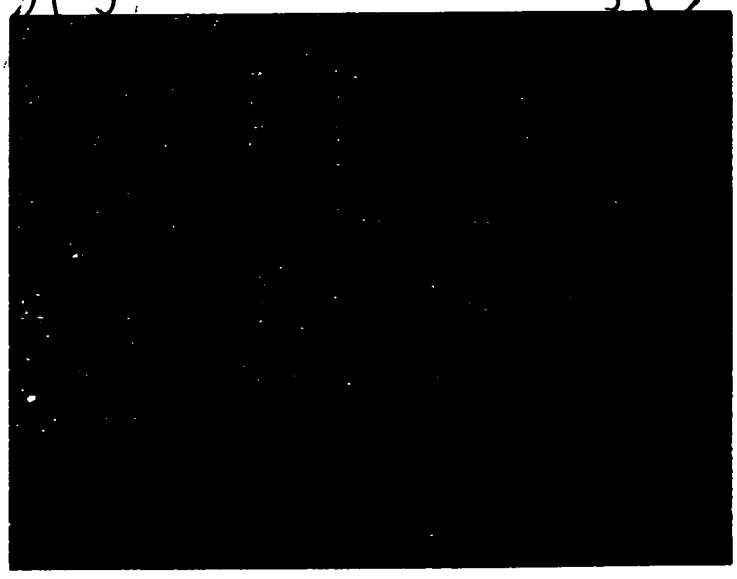
C-test (2nd) on FV(0.9), TN(3.0) 3/[illegible]/18

R	S	T
FV(0.9) 3	FV(0.9) 4	FV(0.9) 5
20	10	10
R→	10	10

U	V	W
TN(3.0) 5	TN(3.0) 3	TN(3.0) 4
20	10	10
V→	10	10

6↗ 2% SDS
15↗ Dye
Add 3.5↗

FV(0.9) 3↗ 5↖ TN(3.0) 5↗ 3↖



Negative

Ligation of FV.9Kb (DdeI), FV.5Kb (H3), 12.1 1.5Kb (H3) 3/5/8

1									
2	FV	DdeI	0.9Kb (Blunt-ended)						
3									
4	DNA		5λ						
5	Vector	(PTZ19R HincII)	.5λ	(50ng)					
6	(10x Lig		1λ						
7	ATP)		1λ		Didn't seem to work				
8	T4		1λ						
9	H_2O		1.5λ						
10			10λ						
11	FV	0.5 H3							
12									
13	DNA		6.5λ						
14	Vector	(PTZ19R H3)	1λ						
15	10x Lig		1λ						
16	ATP)		1λ						
17	T4 Lig		.5λ						
18			10λ						
19									
20	12.1	1.5 H3	(after reprecip)		(3/6/8)				
21									
22	DNA		5λ						
23	Vector	(PTZ19R H3)	1λ						
24	10x Lig		1λ						
25	ATP		1λ						
26	H_2O		1.5λ						
27			10λ						
28									
29									
30									
31									

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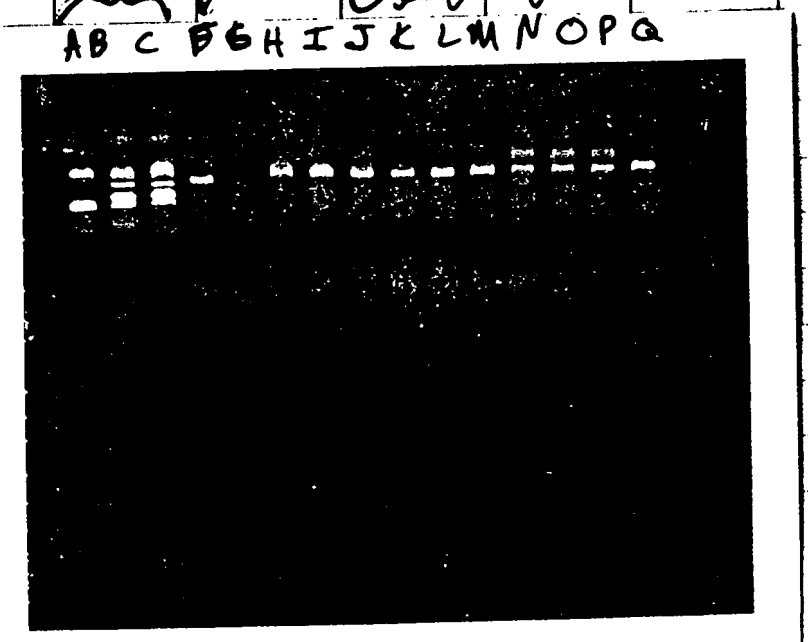
C-tests FV,5H3 FV.9DdeI, TN3.0BglII 3/13/8

	1	2	3	4	5	6	7	8	9
1									
2	A	B	C		D	E			
3	FV(.5)	FV(.5)	FV(.5)		FV(.5)	FV(.5)			
4	III	IV	VI		IV	II			
5	2µl	10	10		2µl	10			
6	A→	10	10		D→	10			
7									
8	F ✓	G	H	I	J ✓	K ✓			
9	FV(.9)	FV(.9)	FV(.9)	FV(.9)	FV(.9)	FV(.9)			
10	1	2	3	4	5	6			
11	2µl	10	10	10	10	10			
12	1→	10	10	10	10	10			
13									
14	L ✓	M	N ✓	O	P	Q			
15	TN3.0	TN3.0	TN3.0	TN3.0	TN3.0	TN3.0			
16	1	2	3	4	5	6			
17	2µl	10	10	10	10	10			
18	1→	10	10	10	10	10			
19					FV 0.9 H3 III IV VI	✓	✓	✓	
20	Add	3.5λ	of Mix						
21			18λ						
22			45λ						
23	vortex, cfg		65C 1h						
24									
25	Run 0.9% gel								
26									
27									
28									
29									
30									
31									



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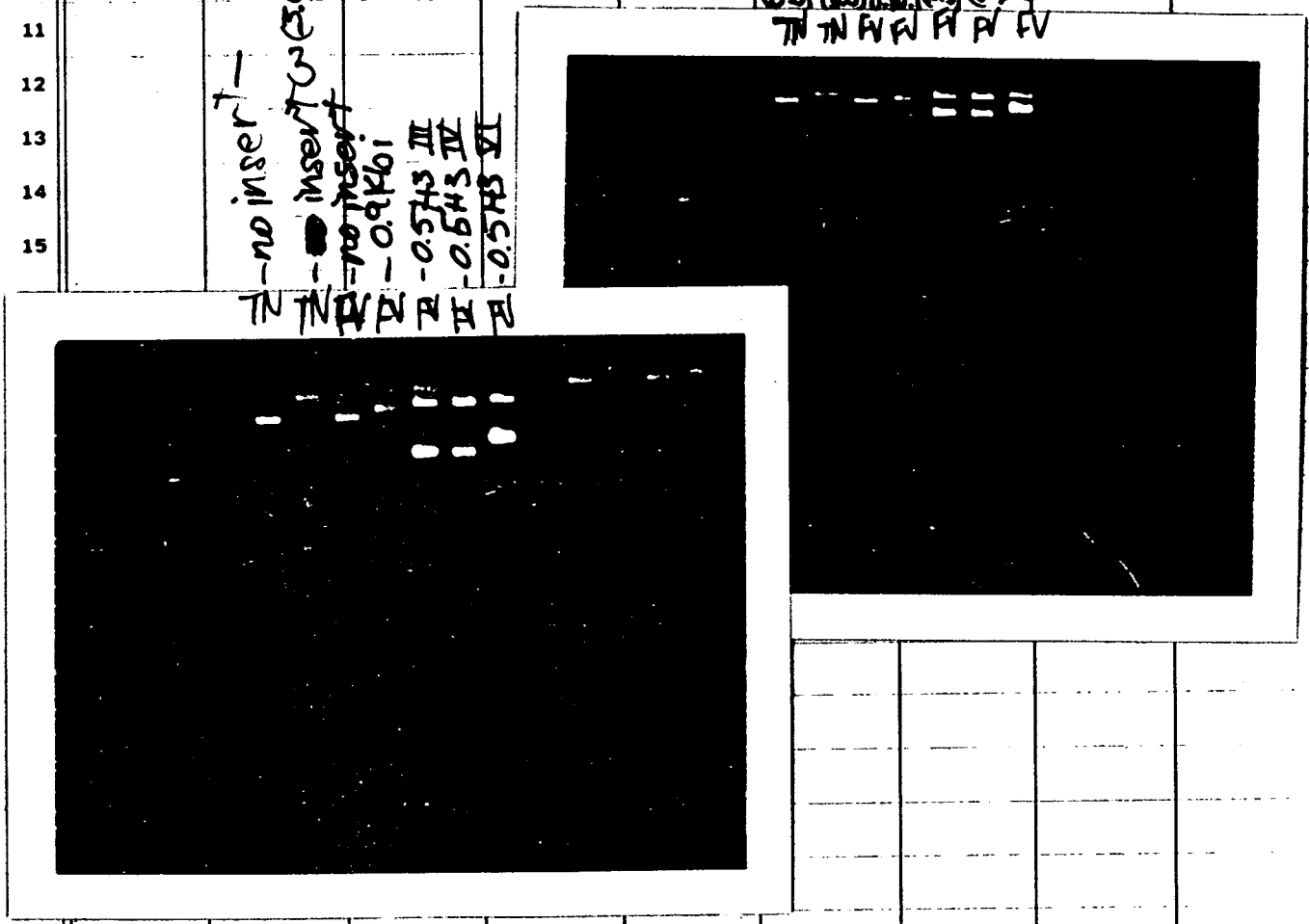
Purified DNA TN(3.0), FV(0.9), FV(0.5) 3/13/18

17



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Sequencing FV.5 Ib, IIb 47.4 XIV, 12.1

3/22/8

FV(5) - re-transformed mini-prep DNA. Picked white colony (DH5αF') Ib and blue colony IIb PTZ19R H3 (C-test strange)
*Still saw shadow bands, but ~~was~~ was able to obtain enough data to make oligo's

12.1(1.5) - portion of XD-1 clone (87.15 region) W/O Bam polymorphism
PTZ19R H3
T6-1's (C-test strange) I, II, IV

47.4 (0.3) - rerun of opp. orientation - something wrong ∑ clone
Hc/Pvu PTZ DH5αF'

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Sequencing Region 47.4

3/15/18

47.4 0.3 Hinc/Pvu (5') - XIII, XIV +12
FV (0.9 Dde I) - 5 m13
TN (30 Bgl II) - 3 m13
FV (0.5 H3) - III, IV, VI PTZ

Annealing mix

① Primer (universal) 8λ
10x TM 6λ

② Primer (reverse) 14λ
10x TM 10.5λ

13λ per 10ml Acryl mix

50ml in beaker - thin lipped Add 65λ Temed, APS
10ml 2.5mix - 13λ Temed, Aps
10ml 0.5 mix - " " "

10mM Tris	40 µl	
Klenow	4 µl	
S^{35}	36 µl	
Template	2µl	spin
Dideoxy>	2µl	spin
Mix	2µl	spin

FV .5 III IV
VI 6 0.5

*Loaded
1 FV (5) VII TCAG

2 FV (9) 5
5 TN (3.0) 3
4 47.4 (0.3) XIII
3 FV (.5) XIV
III
4 IV

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